THE BIOLOGY OF *PEMPHIGUS SPYROTHECAE* GALLS ON POPLAR LEAVES

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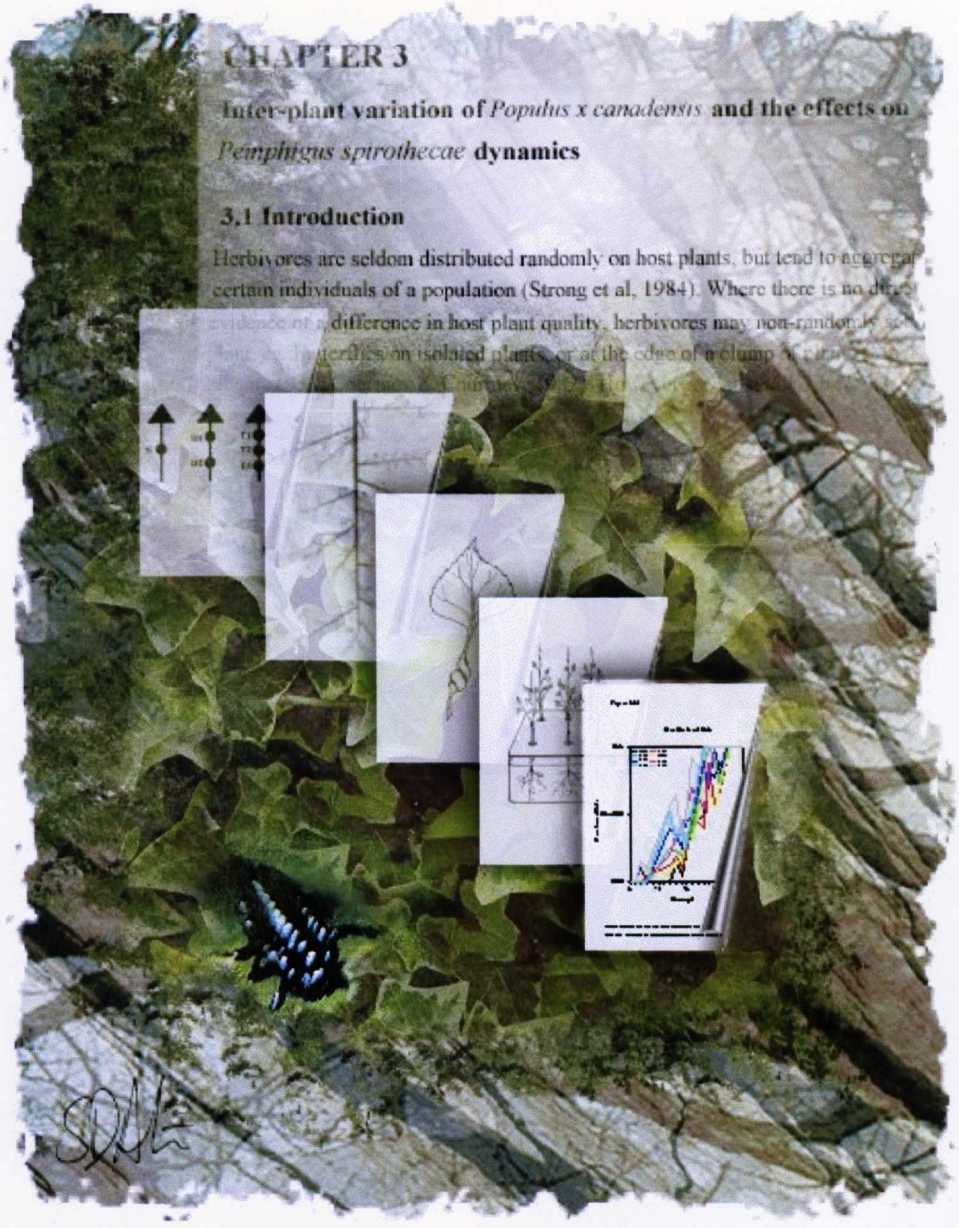
For Steve, Sam and my Parents



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ABSTRACT

The gall forming aphid *Pemphigus spyrothecae* is a plant parasite that colonises the leaf petiole of the black poplar *Populus nigra* and its hybrids and varieties. Models of habitat selection are described and discussed in context with galling aphids. Habitat quality and aphid fitness can be quantified easily. Measures of relative reproductive fitness may be used to determine selection pressures driving habitat selection. The poplar trees differed significantly in budburst phenology, but *Pemphigus spyrothecae* stem mothers hatched on all trees within two days. Structural differences among and within trees were examined. Within and between host plant variation accounted for a dramatic difference in aphid fecundity. Larger leaves or shoots supported galls containing a higher number of offspring and a lower rate of gall failure. On singly galled shoots, gall position on a shoot did not affect reproductive outcome. Most stem mothers located their galls at the top of the petiole, closest to the base of the leaf, but gall position on the petiole did not affect the number of offspring produced. No relationship between stem mother size and fecundity could be detected. When competitor density increased on the same shoot or petiole, average fitness declined. Gall order on the petiole affected the growth rate of the gall tissue, but not the enclosed colony. However, distal galls (furthest from the leaf) were, on average, heavier and had more offspring than proximal galls. Predators are known to be important in regulating insect populations. Although, predators, on average, approached larger galls more often, this was not statistically significant. Due to the restrictive assumptions of models based on the Ideal Free Distribution, no qualitative fit of the distribution pattern of *Pemphigus spyrothecae* aphids was attempted. However, the characteristics of this aphid system were found to approximate an unequal competitors model. The availability of closed leaves on shoots at budburst, when stem mothers emerge, appeared to be the most important factor (of those examined) explaining the population distribution seen in spiral galling aphids.

Chapter 1

INTRODUCTION

1. 1 Habitat selection

The theory of habitat selection has been the focus of much research because it makes predictions about some of the most basic of ecological principles: the spatial distribution and density of organisms. Why animals are found where they are is one of the major questions in ecology. Habitat selection is also relevant in conservation issues since the predictions relate population densities to habitat quality (van Horne, 1983).

Habitat selection has been used to predict the foraging behaviour of animals (Krebs & Davies, 1987) as well as the expected intensity of density-dependent population regulation and dispersal (Morris, 1987, 1988). Habitat heterogeneity influences both population dynamics and demography (Levin, 1976; Adler, 1987) and the pattern of habitat occupation determines the segregation and structure of populations which may influence rates of evolution (Holt, 1987). Habitat selection may also act as a template for the evolution of other ecological strategies (Southwood, 1977, 1988) and differential habitat selection plays a major role in the structure of ecological communities (Schoener, 1974; Rosenzweig, 1987).

Habitat utilisation is not only influenced by morphological, physiological and behavioural adaptations which restrict habitat expansion, but is also constrained by the density-dependence of the demands of other individuals (Grant, 1975; Rosenzweig, 1979, 1981, 1986; Pimm & Rosenzweig, 1981). Factors such as mate choice (Rosenzweig, 1979), predation (Kotler, 1984; Price, 1984) and biogeographical and historical constraints also modify habitat selection. However, there is no doubt that the effects of intra- and interspecific population densities have attracted the most interest from evolutionary and behavioural ecologists.

To understand how populations are distributed in nature, it is important to first consider individuals, since both the micro- and the macro-distribution of populations may be determined by individual decisions. When modelling the distribution of individuals, it is assumed that natural selection will have given rise to genes furnishing individuals with behavioural rules which enable them to optimise their choice of habitat. Since the success of genes is often difficult to determine it is also assumed that an individual's success may be an accurate measure of the success of the genes which program it (Tregenza, 1995). The presence of other individuals is often the main force influencing which part of the environment provides the best conditions in which to maximise fitness components (Maynard Smith & Price, 1973; Parker & Sutherland, 1986), such as mating frequency (Parker, 1978), prey intake rate (e.g. Harper, 1982; Holmgren, 1995) or other aspects (Milinski & Parker, 1991). Animals may affect each other's success through various density dependent processes: exploitation competition, whereby resources are reduced; interference, where factors such as wasting time in interactions with other foragers or disturbing prey decreases fitness; and the influence of density on predation risk (Tregenza, 1994,1995). Therefore, animals are likely to be distributed not only in relation to the resources they require, but also in relation to their competitors.

1. 2 Models of habitat selection

Several models have been formulated to predict the optimal distribution of competitors with some density dependent relationship between competitor numbers and respective fitness in a particular part of the environment (e.g. Parker & Sutherland, 1986; Goss-Custard, 1980; Rosenzweig, 1981). The most successful class of such models can be collectively described as 'ideal free distribution' (IFD) models since they are all based on a few key assumptions described by Fretwell & Lucas (1970) in the original IFD model.

1. 2. 1 Ideal Free Distribution

The 'ideal free distribution' model outlines the distribution of animals as being 'ideal' (individuals will occupy the patch where their resource intake rate is highest), and

'free' (individuals are able to enter any patch on an equal basis with other inhabitants, without any restrictions or costs in terms of time and energy). The main element of Fretwell and Lucas's theory was that the 'suitability' of a particular part of the environment will decrease with an increase in the density of individuals occurring there. Therefore, if the environment contains several patches of different qualities, newly arriving competitors will initially occupy the best patch. However, as the population density increases, the increased use of limited resources will enable individuals to colonise previously poorer patches which now have become equally suitable. This process occurs across the whole environment and leads to an equilibrium at which all patches are equally suitable (Fretwell & Lucas, 1970; Rosenzweig, 1974, 1981; Morris, 1989), a situation caused by different densities of individuals occupying them.

The IFD was originally a simple model to describe how organisms competing for limited resources should distribute themselves according to the suitability of the habitat. This model, despite the restrictive assumptions (Table 1.1, from Tregenza, 1995), has nevertheless found qualitative support from many authors (see Milinski & Parker, 1991; Kennedy & Gray, 1993).

1. 2. 2 Further development of the IFD model

At around the same time as Fretwell & Lucas's (1970) IFD model was introduced to the scientific community, other authors were reaching similar conclusions: Orians (1969) predicted that the number of females on a male's territory was related to the level of resources occurring there; as the best patches become crowded the average reproductive success reduced, and so animals in lower quality patches did not necessarily have lower fitness. Brown (1969) studying great tits *Parus major* also came to similar conclusions. He conceived that as there is an ever-expanding range of habitats, and to optimise maximum production, some individuals are required to breed in relatively poor habitats, and that both poor and good habitats will be used in relation to their value.

It is evident from Table 1.1 that for all the key assumptions of the original IFD, there are many situations in the real world where these assumptions will be violated. By using the IFD model as the theoretical basis, theoreticians have attempted to address the problem of unrealistic assumptions through the development of a range of models, each addressing one or two assumptions of the original theory. Rather than removing one assumption after another to produce a more accurate model reflecting the real world, different authors have used the original IFD and then changed that aspect which they consider most wanting (Tregenza, 1995). Since Fretwell & Lucas's original IFD, numerous models based on the IFD have been proposed to explain population distributions. A detailed description of each model is not within the scope of this thesis, but a brief review of the major models follows. In-depth critiques with quantitative examples of the subsequent theories can be found in Parker & Sutherland (1986), Morris (1989), Kennedy & Gray (1993) and Tregenza (1995) and references therein.

1. 2. 3 Continuous input model - equal competitors

The simplest testable model uses a single aspect of habitat suitability, and some linear density dependent effect on it, for instance the intake rate in a patch and animals scrambling to obtain as large a share of resource items as possible (e.g. Parker & Stuart 1976; Parker, 1978; Milinski, 1979). If patches consist of areas where resource items arrive continually, and are consumed at the same rate as they arrive, e.g fish feeding on food items dropping onto the surface of a pool, or males competing for females arriving at a particular patch (e.g. Parker, 1970), then an increase in competitor density will have a proportional effect on the intake rate. An increase in competitors reduces individual gain rates because the resource items arrive at a fixed rate, until the average gain rate becomes so low that the individual will achieve a higher gain on a lower quality patch subjected to less competition (Sutherland & Parker, 1992). If this process occurs over the entire habitat, an evolutionary stable state (ESS) (Maynard Smith, 1982) is reached where no individual can gain any better by colonising another patch, i.e. all individuals have equal gain rates regardless of which patch they occupy. This state is known as 'continuous input', and gives rise to the 'input matching rule', where the number of competitors in a patch should be

proportional to the total input received by that patch. Although many examples support this rule (e.g. male dungflies matching the arrival of females to a cowpat - Parker, 1978; grazing catfish distributing themselves so that the standing crop of algae in pools receiving different light levels is the same - Power, 1983), others contradict (e.g. the mating success of lovebugs increases with size and position in the swarm, where larger male lovebugs *Pleica nearetica* are at the bottom of the swarm closest to the females - Thornhill, 1980). Input matching can take place over time as well as space. Parker & Courtney (1983) and Iwasa *et al* (1983) found that the emergence of males of two species of butterflies matched the rate of female emergence.

1. 2. 4 Interference model - equal competitors

If animals have to search patches for dispersed resources, the presence of competitors may hinder the rate at which resources can be detected. This form of competition is called 'interference' and can be defined as 'a slowing down in an individual's rate of prey capture due to interactions with other predators' (Goss-Custard, 1980). The patches differ in the density of resource items, and it is assumed that (at least in the short term) competitors exert a negligible effect on resources. If competitors have no effect on each other's rate of intake, then an ESS consists of all the competitors using the patch with the greatest resource abundance. The level of interference will only affect the equilibrium distribution when time wasted in interacting with competitors reduces gain rate, lowering patch profitabilities and causing dispersal of competitors onto poorer quality sites (Sutherland, 1983; Sutherland & Parker, 1985). Examples of interference studies include the most competitive herring gulls *Larus argentatus* (Monaghan, 1980) and oystercatchers *Haematopus ostralegus* (Ens & Goss-Custard, 1984; Goss-Custard *et al*, 1984) aggregating in the best sites.

1. 2. 5 Ideal despotic distribution

The ideal despotic distribution (IDD) was introduced by Fretwell (1972) to describe the expected distribution of territorial species, violating the 'free' assumption of the IFD. The first individuals to colonise a patch establish a territory and exclude any newcomers. Subsequent arrivals are forced to choose between a small territory in the

unoccupied part of the better patch, or a larger site in a poorer patch. For instance, the speckled wood butterfly *Pararge aegaria* takes part in contests for sunlit patches which are usually won by the resident individual (Davies, 1978), and shrews *Sorex araneus* already occupying territories have a competitive advantage over non-residents (Barnard & Brown, 1982). As the density of occupants in a patch increases, so does the advantage of holding a territory, making it more difficult for newcomers to settle, e.g. Patterson (1985) found a high density of convict cichlids *Cichlasoma nigrofasciatum* in better quality habitats, but also that some individuals were prevented from entering these areas. The IDD's only real prediction is that otherwise equal competitors will differ in success rate if some individuals hold territories and some do not. The IDD is a useful tool for discussion, but for the same reasons as the original IFD, can only approximate any real situation.

1. 2. 6 Unequal competitor models

Individual animals within a population are unlikely to be equal in competitive ability. There may be short-term implications for population distributions such as individual age or behavioural strategy, that lead to short-term differences in competitive ability, or differences may be long-term, with some phenotypes superior to others. Sutherland & Parker (1985, 1986, 1992) and Parker (1982) use a model where there are two patches and two phenotypes, which are given 'competitive weights'; a measure of their relative competitive ability. A phenotype with twice the competitive weight of another will obtain twice the uptake rate in the same patch, with the individual's uptake rate related to its competitive weight relative to the mean or total competitive weight in the patch. Two models based on unequal competitors have been developed by Sutherland & Parker (1985, 1992), namely competition through exploitation of continuous input resources and competition through interference. Rosenzweig (1974, 1981, 1986) developed a similar model, called the isoleg theory. Competitors are considered to be two different species each with a different competitive ability, and the habitat contains two patches of different quality. The isoleg model is different from unequal competitor IFD models since it does not make quantitative predictions, but estimates the absolute size of populations, and limits them with an environmental carrying capacity. It predicts that at low competitor density, both species will be found

on the better patch and as the population densities of both species increase, some subordinates will be forced onto the inferior patch, since it is assumed that fitter competitors have a greater advantage on the better patch. If the population density of the dominant species increases further, all subordinates are forced onto the inferior patch, whilst the dominants will only use the substandard patch when their own density rises sufficiently; they cannot be forced there by subordinates since it is assumed their density cannot reach this point. Although models with only two patches are no doubt favoured because of their experimental simplicity, recent research has examined models using multi-patch systems (e.g. Carter & Abrahams, 1997).

1. 2. 7 Kleptoparasitism

In some animals competition can occur through the loss of resources to other individuals. Each competitor has equal prospects of finding a resource item in a given patch but, surrounded by more dominant individuals, is less likely to be able to hold on to the item. For instance, Fulmars *Fulmarus glacialis* holding a piece of fish may be subjected to repeated attacks by conspecifics and will frequently be forced to forsake the fish (Enquist *et al*, 1985). This type of interference is different from other forms of interference since density on a patch does not necessarily affect the average gain rate if competition is only due to kleptoparasitism. Parker & Sunderland (1986) developed a model with a dominance hierarchy of phenotypes and where competitors steal food from their subordinates. Profits and losses to a competitor increase linearly with the number of individuals above and below the individual in the hierarchy. Kleptoparasitism in its simplest form leads to a reallocation of resources between individuals differing in stealing ability.

1. 2. 8 Perceptual constraints model

Most models assume that foragers are omniscient, and that their distribution is related to resource distribution and competition. However, many animals have an imperfect knowledge of patch profitabilities caused by two possible factors: a) foragers may be unable to distinguish between the profitability of similar patches, and b) foragers may be unaware of the profitability of unattended patches. Abrahams (1986) suggested that

the first factor, described as 'perceptual constraint', causes departures from an ideal free distribution. He presented a model in which the aptness of a patch is defined as the total resources in a patch divided by the number of foragers present. Perceptual limitations generally lead to the underuse of better quality patches and the overuse of inferior patches, with greater perception limits leading to greater deviations from an IFD. If there are large differences in patch qualities and the perception limit is fine, more animals are able to determine which is better so there are fewer 'guesses'. However, if perception limits are large, then greater differences between patches lead to a poorer fit to an IFD because guesses are 'more wrong'. The perceptual constraints model differs from an interference model in that it predicts a higher resource intake rate in the better patch, in contrast to the equal competitor IFD, which predicts an equal intake in all patches. However, if competitors vary, which is more likely, a higher mean intake rate in better patches is probably a result of unequal competitors.

1. 2. 9 Patch assessment model

The second factor responsible for violating the assumption of omniscience is that animals cannot know the profitability of patches they have not visited. Foragers must visit all the patches in the environment or at least be able to estimate patch qualities using as many sources of information as possible. Harley (1981) developed a simple learning rule called the relative payoff sum (RPS) rule, which enables animals to decide which patch to choose. An animal chooses a certain behaviour based on how successful that behaviour was in the past. The decisions are then made after a sufficiently long time period allowing for the animal to have experienced payoffs from all possible behaviours. The rule states that the profitability of exhibiting a particular behaviour is equal to the sum of the previous gains from that behaviour divided by the sum of previous payoffs from all possible behaviours. If the rule is applied to patch choice, the forager chooses to search for resources on each patch with a profitability equal to the gains achieved on that patch in the past divided by the gains achieved on all patches.

1. 2. 10 Resource dynamics model

In many models only one source of density dependence has been considered at any one time. Either resources remain at a constant abundance, with competition resulting from interference, or resources are replaced immediately following utilisation, with competition resulting from exploitation. However, Lessells (1995) suggests that interference models can be considered a snapshot of continuous input models. If resources are continuously input into patches, the assumption that they are immediately consumed can be slackened, so that the standing crop (resource items arriving in the patch but not yet consumed) in each patch can be considered. If there are equal competitors, all foragers must have equal uptake rates. With exploitation competition and no interference, the standing crop is equal in each patch, and therefore the resource (prey) mortality rate will be density independent. If interference occurs together with exploitation competition, the intake rate will decrease in the superior patches, and as uptake rates must still be equal, there will be a greater standing crop in those patches. The prey mortality rate now becomes density dependent, increasing with resource input rate, but at a depressed rate, since the standing crop will be greater in patches with higher input rates.

Some patch selection models assume that prey items are immobile. However, this is clearly not always the case; for instance sunfish *Lepomis macrochirus* change their distribution in the presence of predatory bass *Micropterus salmoides* (Werner *et al*, 1983). Sih (1984) suggests that if a refuge exists and prey are mobile, their response will determine the distribution of both species. Schwinning & Rosenzweig (1990) have modelled the distribution of populations between an open habitat and one containing refuges.

1. 2. 11 Survival as a measure of fitness

Another assumption of the IFD states that predators are out to maximise their short-term fitness, measured as the net gain rate of some resource. McNamara & Houston's (1990) model defines fitness in terms of long-term survival during a non-reproductive

period rather than relative to the rate of energetic gain. The variability of resource items and the risk from predation when foraging are important in terms of fitness and must be considered when attempting to predict animal distributions. Foragers are assigned reserves, and below and above certain thresholds the animal either dies of hunger or rests. Foragers have a choice of two patches, but no cost is involved in switching between patches. The behaviour of a forager is determined at any point by using a dynamic programming technique. This allows an animal's optimal behaviour to be found by retracing it back in time. The forager's energy reserves can be determined and from this the optimal patch choice. This model, applied to a range of patch types and both individuals and populations is known as risk sensitive foraging (see review by McNamara & Houston, 1992).

1. 3 Applying theory to real situations : galling aphids as a model system

Applying ideal free type models to population dynamics is not as straight forward as it may seem. Even those cases chosen because they approximate the assumptions of a theory do not in reality demonstrate a confident level of support for the predictions of the models they test. Maybe the model used is unsuited to the animal system studied, or perhaps researchers have incorrectly identified the distributions they are studying. Even though both Kennedy & Gray (1993) and Tregenza (1994) detect errors in recent publications modelling IFD's, the second explanation is unlikely. In real-life situations there are many problems associated with the study of animal distributions, for instance it is often difficult to assess the value of resources; several different resources may be utilised at the same time, so the one investigated may not be the primary factor; competition may take place in different ways each requiring a different theoretical approach; there are inherent difficulties in monitoring subjects 24 hours a day, optimal distributions may be achieved over both time and space; and it is not always easy to identify the location of all competitors in the field and to assess which animals are being successful (Tregenza, 1995). Kennedy & Gray (1993) argue that the IFD is not sufficiently accurate in predicting the distribution of foraging animals. They criticise past studies using competitive distributions and discuss alternative insights on deviations from the IFD theory's predictions. However, other authors (Milinski, 1994; Tregenza, 1995) claim that Kennedy & Gray's paper comprises a series of misunderstandings and much of what they state to be new perspectives have been known for several years.

Because it is extremely difficult to measure individual fitnesses in habitats of known quality, galling aphids constitute an excellent model species, since aphids produce all their offspring in a single gall which can readily be assessed. Even failures to colonise unsuitable patches (fitness equals zero) are evident. Aphids are a common agricultural pest and are responsible for the transmission of many plant viruses, therefore predicting the distribution patterns of aphid populations is of particular value in crop protection (Blackman & Eastop, 1984; Hille Ris Lambers, 1972; Harris & Maramorosch, 1977). Although the life-cycles may not be identical, by using galling

aphids as models for aphid populations in general, it is nevertheless possible to determine how factors such as density dependent competition and predation are important in regulating population density, and therefore to predict to a certain degree of accuracy the distribution of free-living aphids. This study involves the galls of *Pemphigus spyrothecae* Pass. (Pemphigidae) on the petioles of poplar leaves *Populus* x *canadensis*.

Numerous studies have examined insect distribution patterns on host plants (e.g. Mackay & Singer, 1982; Watt, 1990; Memmott, Day & Godfrey, 1995), but in particular Whitham and his co-authors (e.g. Whitham, 1978, 1980, 1981; Larson & Whitham, 1991) have attempted to explain how galling aphids select suitable habitats and the subsequent effects on reproductive fitness. Whitham (1980) noted that although several measures of fitness (for instance, both the mean number of offspring per gall and gall failure are equal on leaves of different quality and competitor density) confirm the original IFD model, the critical assumptions built into the model are violated. Since the IFD does not account for dominance and territoriality in individuals, no coloniser can prevent another aphid from settling and using the available resources. As newcomers arrive in a patch, the expected fitnesses of all individuals decreases by an equal amount, and all have the same expected fitness. Dominance and territoriality in the Ideal Despotic Distribution (IDD) should hinder settling of subdominants and cause expected fitnesses within the patch to be unequal. Whitham (1980) concluded that within a habitat where aphids (e.g. *Pemphigus betae*) display territorial behaviour as documented in Whitham (1979), expected fitnesses will be unequal, and this characterises the IDD rather than the IFD. Another violation of the assumptions of both the IFD and IDD in Whitham's system is the supposition that habitats are homogeneous; in reality most natural habitats are heterogeneous.

When attempting to predict the population distribution of any species, it is important to consider the assumptions of current IFD theories, since there are relatively few examples where models make accurate predictions about the distribution of foraging animals. At present it is best to discuss the areas where the animal system studied violates the assumptions of current models rather than attempt to fit the system to a simple IFD model (Tregenza, pers. com.). Nevertheless, it is still possible to propose

some general predictions about the distribution of galling aphids based on current habitat selection theories.

In the ideal free distribution all competitors are assumed to be equal and each is free to enter the patch where the gains are highest. Given that galling aphids exhibit dominance behaviour, and hence are of unequal competitive ability, the most appropriate models are those of unequal competitors developed by Sutherland & Parker (1985, 1986, 1992). However, even these models are unlikely to provide any concrete predictions because they are based on the original assumptions of the IFD (Table 1.1), which are unrealistic in most natural situations. Therefore no quantitative fit is attempted in the present study. The unequal competitors theory assumes that an increase in population density leads to a decrease in gain rate through mutual interference, and that individuals differ in competitive ability. The main predictions are that more than one phenotype cannot mix across two patch types (termed 'truncated phenotype distribution'), i.e. only individuals with adjacent competitive abilities (ranked in increasing ability) may coexist in the same patch type; that higher quality patches contain the most competitive phenotypes; and that fitness is correlated with both phenotype and patch quality. A measure of competitive ability in a galling aphid may be defined as the size of the colonising first-instar stem mother. In territorial interactions the largest *Pemphigus betae* foundress usually wins or successfully defends the favoured basal position on the leaf blade (Whitham, 1979), where they achieve the greatest reproductive success (Whitham, 1978). The predictions from the unequal competitors model may then be interpreted such that only similar-sized fundatrices would initiate galls in the same type of patch; that the largest fundatrices would gain access to the most suitable habitats for galling, say on the largest leaves of a host plant, since the amount/quality of nutrients translocated to and from the leaf is correlated with leaf size (Whitham, 1978); and that fitness (for instance reproductive success or mortality) would be correlated to leaf size and the size of the stem mother.

1. 4 Biology of aphid galls

Among gall-forming insects, the aphids are unique in many ways, as they have evolved complex life cycles and are often host specific. Aphids use the gall as a nutritional and protective environment and as an incubator in which to reproduce parthenogenetically hundreds or even thousands of offspring before dispersal takes place (Wool, 1984). Gall-forming aphids belong to three sub-families of the super family Aphidoidea, although within these families not all genera or species initiate galls. Aphid taxonomy has in the past been confused due to the complex life history of aphids. Different morphological forms of the same species described on different host species and in different parts of the world were sometimes given species or generic status. For instance, within the genus *Forda*, *Forda marginata* has 16 invalid synonyms (Eastop & Hille Ris Lambers, 1976). Therefore the figure given by Mani (1964) of over 700 species of gall-forming aphids may be an overestimate. The taxonomy of aphids may also be complicated by the mere definition of a gall. Many species cause leaf or stem deformations which have previously been referred to as galls, although the aphids have not been completely enclosed within the gall. These are now known as 'pseudogalls'.

Gall-forming aphids are found on all continents except Antarctica. Many aphids complete their life cycle (holocycle) on two hosts, with galls appearing only on the primary host. Therefore, the distribution of galls may be limited to the distribution of this host. However, forms living on the secondary host may have a wider distribution and if the primary host does not occur locally, may live without ever producing galls (anholocycle) (Hille Ris Lambers, 1957). The primary hosts of Fordinae do not grow naturally in America, but several species live anholocyclically on the roots of grasses (Wool, 1984). The cultivation of ornamental and other trees has led to the transfer of such species between countries and continents. This has resulted in the occurrence of aphid species in areas in which they were previously unknown. For instance, *Tetraneura ulmi* and other forms have been introduced in North America. These are most likely to have originated in Japan (Hille Ris Lambers, 1970).

Aphid galls vary in size and shape: certain species of Phylloxeridae, Fordini and *Thecabius* produce small, round pocket-shaped galls; Adelgidae form a many chambered cone-like gall; Baizongiini galls are elongate, globular or branched structures; most species of *Pemphigus* make globular or rounded galls on the petiole of *Populus* (Wool, 1984); and *Mordwilkoja vagabunda* on *Populus* and *Slavum wertheimae* on *Pistacia* have complex coral-like galls (Hille Ris Lambers, 1957). Mani (1964) suggests that the complexity of gall shapes reduces as the plant tissue age increases. Galls house one to thousands of occupants. A gall of *Astegopteryx styracicola* can frequently have over 200,000 inhabitants (Aoki, Yamane & Kiuchi, 1977).

Most galls are produced on growing shoots, young leaves or plant roots. Stimulus for gall formation is thought to be caused by the colonising aphid and this appears to be species specific. Different aphid species form different shaped galls on the same host. For instance, *Pemphigus spyrothecae* and *Pemphigus bursarius* both form galls on the petiole of poplar leaves, but the first induces a spiral-shaped gall formed by the twisting of the petiole, whereas the latter forms a hollow purse-shaped outgrowth from the side of the leaf petiole (Dunn, 1960) (Fig. 1.1). The exact substance which causes the gall remains a mystery. Aphid saliva contains many compounds, some of which could be cecidogenic (Miles, 1972). The two most likely substances are the plant growth hormone IAA (Indole -3- Acetic Acid, either transmitted in the saliva or formed in the plant by oxidative transamination of tryptophan by enzymes in the saliva) or free amino acids in the aphid's saliva (Miles, 1972). The active substance may be similar in all Hemiptera, and the eventual gall structure may merely depend on the site and stage of plant tissue attacked, or on the feeding behaviour of the aphid (Dunn, 1960).

The state of the plant is often vital to the success of the attack by the aphid. In Pemphigidae, only the primordial leaves at bud burst are responsive to gall formation. In the Adelgidae the aphid attacks the terminal bud of *Picea* in autumn and overwinters with its stylet inserted into the plant tissue. Its success in forming a gall the following spring depends on reaching the correct bud tissue (Rohfritsch, 1981). Two types of gall are formed by *Pemphigus populitransversus* on *Populus deltoides* at

different times of the year. Elongate galls are formed early in spring on young leaf petioles, and globular galls are induced later in the season (Faith, 1979). Not only does this dimorphism affect gall shape, but also reproductive outcome, as the larger, later leaves support larger galls containing a greater population size than the smaller elongate galls. In some *Pemphigus* species, gall development ceases if the fundatrix dies before completing the gall, and only a scar remains visible. *Baizongia pistaciae* galls grow for several weeks before attaining maturity, while others such as Fordinae and Pemphiginae galls are completed within a week or two.

A typical complete life cycle of a Pemphigid aphid involves migration from a primary host on which the gall is formed onto a secondary host without the production of a gall. Emerging from an egg produced by the sexuals on the primary host, the nymph migrates out in early spring onto newly unfurling leaves and begins to feed (Dunn, 1960). A gall is initiated and the fundatrix matures into an apterous adult reproducing parthenogenetically. In most *Pemphigus* species, the all-female fundatrigeniae (offspring) mature into alate forms and disperse in midsummer onto their secondary hosts, where they larviposit. The new generation (radicicolae) penetrate the soil and feed on the grass roots. At the end of summer a new alate form is produced (sexuparae). These fly back to the primary hosts and give birth to sexuals (males and females) which mate and produce the overwintering eggs. There are many variations to this generalised holocycle. In some Fordinae, the radicicolae overwinter on the secondary host and the sexuparae are produced the following spring, and so this holocycle lasts two years rather than one (Bodenheimer & Swirski, 1957).

Some gall-forming aphids do not have a secondary host, and the sexuparae produce the sexuales on the primary host. For instance, *Slavum wertheimae*, *Pemphigus monophagus* and *Pemphigus spyrothecae* are such monoecious species (Hille Ris Lambers, 1957). In other species, two galls are produced on the primary host; the fundatrix produces a primary gall on the leaf midrib, and the apterous offspring migrate to the leaf margins where a final gall is formed (Bodenheimer & Swirski, 1957). There are other deviations from the general life cycle described above in groups such as the Adelgidae and the Phylloxeridae, and a detailed description of their life cycles can be found in Carter (1971).

Most primary hosts are deciduous trees (except Adelgidae on evergreen Pinaceae), and the galls are therefore lost in the autumn with leaf drop. An interesting exception is *Morwilkoja vagabunda* on *Populus*. The galls remain attached to the branches and appear to attract the migrating sexuparae. These enter the abandoned galls and produce the sexuals within the shelter of the gall (Ignoffo & Grabovsky, 1961). The empty galls of *Aploneura lentisci* on *Pistacia lentiscus* are not shed in autumn, but house a number of inquilines such as spiders, mites and scale insects, and another free living aphid, *Aphis* (Koach & Wool, 1977).

The rate of infestation of some galling aphid species can be high on a host tree. Nutrients are diverted for gall formation and clone growth, and this has a detrimental effect on the host plant (Ignoffo & Grabovsky, 1961). *Baizongia pistaciae* inhibits branch growth and *Pemhigus populivenae* causes chlorosis of the leaf distal to the gall (Whitham, 1978). Although galls of *Phylloxera* species damage cultivated pecan varieties, in most cases it is the free-living forms on the secondary hosts that account for the commercial damage. Common examples are *Pemphigus betae* on beet in Canada, *Pemphigus bursarius* on lettuce in England, Eriosomatinae on pears in Yugoslavia and the famous grape pest *Phylloxera* (Schaefer, 1976).

Galls may represent an adaptation of the plant to the attack by the aphid (Wool, 1984). The damage to the plant is localised at the point of the gall, and is likely to be far less than the equivalent number of free-living aphids. Gall formation is advantageous to the aphid: while other tissue parts soon become unpalatable, within the gall, food and shelter are provided throughout the season. Humidity is close to air saturation and this may be an important factor for aphids during a dry and hot summer. Temperatures also appear to be milder than the immediate areas (Wool, 1984). It seems that a gall is an ideal choice for a nursery.

1. 5 Biology of *Pemphigus spyrothecae* Pass. (1860)

The initiation of galls on *Populus* trees in spring is general to all *Pemphigus* species. The primary host in the British species of *Pemphigus* is *Populus nigra* Linnaeus and its varieties and hybrids (Dunn, 1960). Several different *Pemphigus* species appear on the same tree, and may form galls on the same leaf. *Pemphigus bursarius* L. initiates a pouch gall on the petiole, *Pemphigus spyrothecae* Pass. and *Pemphigus protospirae* Lichst. form spiral-shaped petiole galls, and *Pemphigus filaginis* Fonsc. produces a wart-like gall near the midrib of the leaf.

The aphids of the Pemphigidae have a relatively simple holocycle. They account for approximately 7 % of all aphid species (Foster & Benton, 1992). In autumn, winged females, the sexuparae, fly to the primary host on which they do not feed. *Pemphigus spyrothecae* sexuparae are produced in the galls on the primary host (poplar) and so do not have far to move to the bark (Fig.1.2). They then walk from the outer, smaller branches down to the trunk, in many cases more than 1 metre, and settle in a bark crevice, where they produce a small number of both males and females (the sexuals) (Fig.1.2). Males are usually born before females (Foster & Benton, 1992). The sexuals mate and each female produces one egg (Fig.1.2) laid deep in a crevice in the *Populus* bark. At budburst in spring the aphid stem mother (fundatrix) (Fig.1.3) hatches and migrates from the bark down to a young leaf shoot. The females may walk several metres along branches until a suitable shoot is located.

The formation of a gall depends on the petiole cells' ability to divide and expand and therefore older petioles, past the general growth flush, are not used. Once on the petiole the stem mother inserts her stylet in a zigzag pattern, moving slowly over a small area (Dunn, 1960). The stylet can be inserted in the same position for up to 10 minutes or more. Growth is arrested at the insertion site, causing the petiole to bend towards this area since growth continues as normal on the opposite side of the petiole. Further stylet insertions cause twists in the petiole, resulting in three coils being formed, enclosing a space within which the fundatrix settles. The fundatrix then stimulates the gall to swell, increasing the size of the internal hollow. Should she die prior to completion of the gall (up to seven days), no further growth is seen (Dunn,

1960). Failed or aborted galls can be seen as a kink in the petiole or as an empty incomplete gall. In previous studies by Whitham (1979), *Pemphigus betae* aphids have been shown to be territorial on the leaf midrib, and compete in kick-shove contests to determine who occupies the base of the midrib: a similar behaviour occurs in *P. spyrothecae* (pers. obs). In some instances several stem mothers may successfully form a gall on the same petiole.

Within the gall the fundatrix (first generation) parthenogenetically produces thick-legged first-instars which mature into wingless virginoparae (second generation) (Plate 1.1 and Fig. 1.3). These give birth to both thick-legged (termed soldiers) and normal-legged first-instars that turn into winged sexuparae (third generation) (Foster, 1990). From early August to late October, the winged aphids emerge through the ostiole, a furrow between two of the coils, and fly back to the trunk, where they produce the sexuals, and another cycle begins (Fig. 1.4). The males fight for access to females and do not discriminate between sibling or non-sibling opponents, nor between related or non-related females (Foster & Benton, 1992).

The gall community also includes inquilines such as spiders, mites, psyllids, scale insects and the cohabiting aphid, *Chaitophorus leucomelas*. In empty galls of *Aploneura lentisci* an *Aphis* sp. was found surviving and reproducing. This can only happen in this species since galls are formed on the evergreen *Pistacia lentiscus* and galls remain on the plant for months and are succulent enough for the aphids to live in (Wool, 1984).

1. 5. 1 Soldiers

Pemphigus spyrothecae aphids are especially interesting as the gall colony also contains a soldier caste. Soldiers are known from two aphid families, the Hormaphididae and the Pemphigidae. A soldier is regarded as an individual acting defensively despite the likelihood of reduced fitness to itself (Foster & Northcott, 1994): Foster (1990) found an average of between 9 and 30 soldiers died when a predator was introduced into the gall. Hamilton (1964) described the evolution of

altruistic behaviour as an outcome of kin selection, since closely related organisms are more likely to behave altruistically. Although aphid soldiers behave in an altruistic way, there is no evidence to suggest that kin recognition exists in aphids (Aoki *et al*, 1991). Recognition is generally based on chemical cues, using hydrocarbons on the surface of the insect's body. Errard (1994) discovered that individual ants could recognise the allospecific cues borne on each individual's body surface, even though the ants had been separated for up to a year and only trace amounts of allospecific hydrocarbons remained present. Aphids may have a less sophisticated discriminatory olfactory capability.

In the Hormaphididae two types of soldier can be found. The first, the horned soldiers, occur on the secondary host e.g. bamboo, and are first instars with thickened forelegs with strong claws, sclerotized tergites, and sharp frontal horns. The second form of soldiers are typically sterile, dimorphic second instars using their stylets and stout spines on their heads as weapons (Aoki, 1987). The production of sterile soldiers is costly. Since mothers reduce the number of fertile progeny, soldiers must compensate by having a strong defensive ability (Sakata & Itô, 1991). In *Pemphigus* aphids, the non-sterile soldiers appear on the primary host. Stylets and hind-legs are most often used as weapons (Foster & Northcott, 1994).

Aphids reproduce parthenogenetically, so a colony may contain a pure clone where all individuals are genetically identical (Foster & Northcott, 1994). Being in a gall helps to maintain the integrity of the clone, but the gall must be opened for the removal of debris and honeydew droplets, and to eventually allow the winged progeny to disperse from the gall. Foster (1990) never observed *Pemphigus spyrothecae* attacking conspecifics or other aphids, for instance the cohabiting *Chaitophorus leucomelas*, and concluded that the soldiers' primary role was to defend the gall against predators. Since conspecific aphids are usually unharmed, intergall migration may make a pure clone unlikely. To measure relatedness of aphids in a gall, biochemical and genetic techniques are required.

Benton & Foster (1992) observed the housekeeping role of the *Pemphigus spyrothecae* soldier caste. The soldiers remove cast skins and dead aphids as well as

honeydew droplets, which are encased in wax to form non-wetting droplets. The accumulation of honeydew may result in potentially harmful fungal infections (Buckley, 1987), and the removal is therefore essential for gall survival. Other authors (Aoki & Kurosu, 1989; Kurosu *et al*, 1990; Aoki, 1980) have also reported gall cleaning behaviour by aphids of both the Pemphigidae and Hormaphididae. Gall cleaning behaviour has also been observed in some aphid species that do not attack predators (Kurosu & Aoki, 1991). Foster & Northcott (1994) speculate that the removal of honeydew may be a precursor of defensive behaviour. When pushing droplets out of the gall, aphids may have encountered predators trying to enter the gall. The removal of honeydew from the gall may also serve to attract ants. Del-Claro & Oliveira (1996) reported the treehopper *Guayaquila xiphias* flicking away accumulated honeydew droplets. These fall onto lower leaves and the surrounding ground and increase the probability of attracting ants, and thus being tended to, and protected from predators. For a full review on the evolution of soldiers in aphids, see Itô (1989) and Stern & Foster (1996).

1. 6 Site Description

The host plants examined in most of the studies described in this thesis are hybrid black poplars (*Populus* x *canadensis*) located at Attenborough Gravel Pits, Nottingham, England, a Site of Special Scientific Interest (SSSI), jointly managed by Nottinghamshire Wildlife Trust and owners Butterley Aggregates Ltd. The reserve, established in 1966, consists of flooded gravel pits with associated marginal and wetland vegetation. The process of recolonisation over some 40 years has created a wide range of aquatic and waterside habitats. Between the ponds are drier areas of scrub and grassland as well as areas of native willow and old stream courses. New species of plants and invertebrates are continually being recorded as part of the recolonisation process. The reserve has a wide range of fish and invertebrates, but is best known for its diverse array of bird species.

The poplar trees face an open car park adjoining a small expanse of water and are thus exposed to the full force of the prevailing wind and weather, but are unlikely to be subjected to very different climatic or environmental conditions. The trees were denoted by the letters A to M, with A being closest to the old entrance to the nature reserve. Aphids were collected from this reserve between 1992 and 1995. Kearsley & Whitham (1989) noted in their investigations on *Populus angustifolia* that plants undergo a change in resistance to herbivory as they mature, with the gall-forming aphid, *Pemphigus betae*, 70 times more common, and twice as likely to survive, on mature rather than juvenile trees. However, in this study, all the selected *Populus* trees were mature and planted at the same time nearly 30 years ago. These hybrid black poplars were already known to support a large population of *Pemphigus spyrothecae*. Other host trees examined were *Populus nigra* var. *italica*, and these mature trees are located on the University of Nottingham campus.

1. 7 Study objectives

I predict that poplar trees are highly heterogeneous environments, and that the quality of trees correlates with *Pemphigus spyrothecae* fitness, and since these aphids are monoecious, I anticipate that they are likely to be adapted to individual host trees. If the largest aphid stem mothers usually win or successfully defend superior habitats in territorial interactions (Whitham, 1979), I expect larger *P. spyrothecae* stem mothers to occupy larger sized leaves which are correlated to heavier galls containing more progeny and to least gall failures. I anticipate *P. spyrothecae* aphids initiating galls on single petioles to have a greater reproductive success than those forced to double up on the same petiole. Galling aphids are subjected to predation from many natural enemies (e.g. Wheeler & Jubb, 1979; Wool, 1984), and I predict that, despite having a soldier caste, predation reduces the reproductive success of *P. spyrothecae*.

1. 7. 1 Study outline

In the first two chapters I test for between- and within-habitat heterogeneity and the effects on aphid distribution patterns and reproductive success. Chapter 4 details the emergence of aphid stem mothers in spring and examines evidence of budburst synchrony and adaptation to individual host plants. In chapter 5, I chart gall growth and the increase in colony individuals as the season progresses. In the next chapter I investigate the effects of intraspecific competition on aphid reproductive success, and in chapter 7, I examine the effect of predation on gall distribution and aphid mortality. The chapters are written as papers to facilitate submission for publication; this entails a certain amount of repetition in introducing the insect system.

Table 1. 1

Assumptions made in ideal free distribution models and the violations of these

Assumption	Potential real situation
Equal competitive abilities	Variation between individuals in: Susceptibility to interference Search efficiency Handling time 'State' (for instance food reserves) Knowledge of resource distribution Cost of travel Resource holding ability Susceptibility to other environmental factors (e.g. predation, temperature) Other priorities (e.g mate searching, kin selection)
Omniscience	Animals may be constrained by: Having to visit patches to assess their profitability Inability to detect the difference between patches Inability to remember patch profitabilities
No costs to travel between patches	Costs to travel between patches in terms of: Time Energy Risks from predation, physical factors
No interference between competitors	Various reversible factors leading to reduced intake rate with increased competitor density including: Mutual interference-wasting time or resources Kleptoparasitism Despotism
Resources are fixed in space and time	Exploitation leads to patches depleting Prey may move in response to predation New patches may arise
Only rate of resource acquisition affects patch choice	Patches have associated costs or benefits, such as: Predation risk Physical properties of the environment (e.g. temperature, rate of flow of moving water) Refuges, nest sites
Distribution is dictated entirely by competitors maximising short-term fitness	Animals may have other priorities, e.g. Mate searching Migrations

From Tregenza (1995)

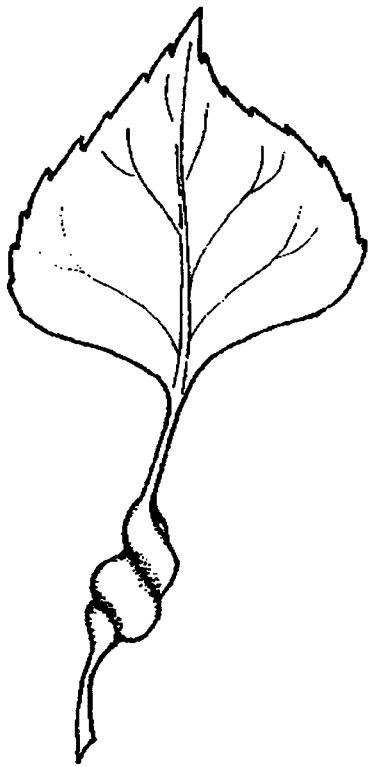
Plate 1. 1

10 mm

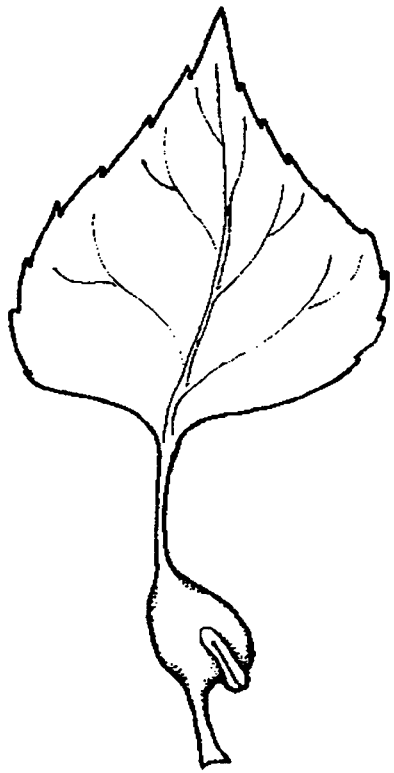
The *Pemphigus spyrothecae* fundatrix produces her offspring within a single gall

Figure 1. 1

Two different galling aphid species on poplar petioles



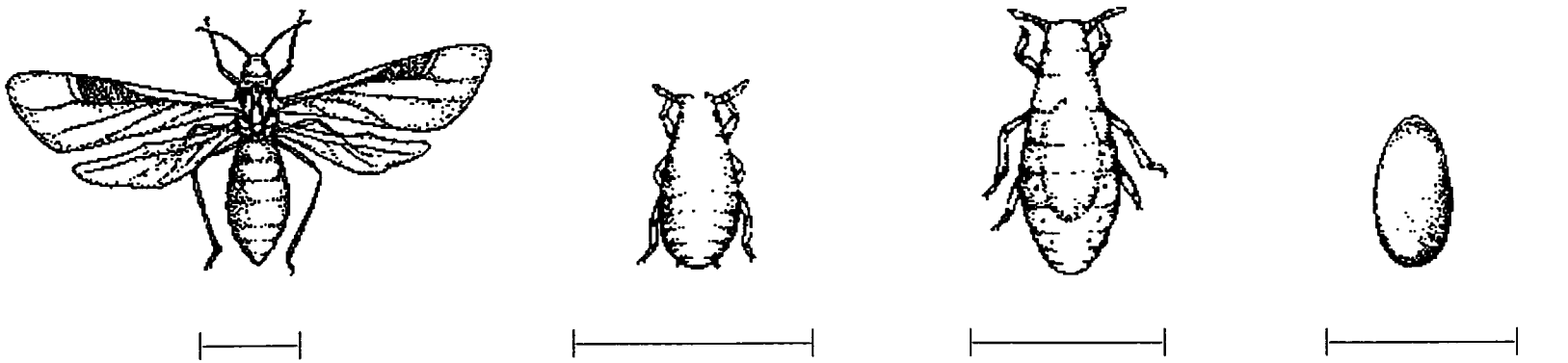
Pemphigus spyrothecae



Pemphigus bursarius

Figure 1. 2

Several generations of *Pemphigus spyrothecae*

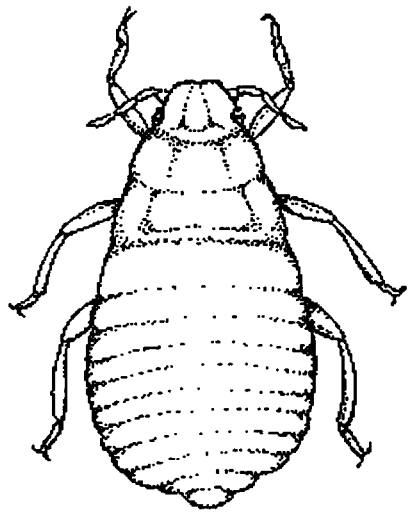


Sexuparae | Male sexual | Female sexual | Overwintering egg

(adapted from Tótn, 1939).

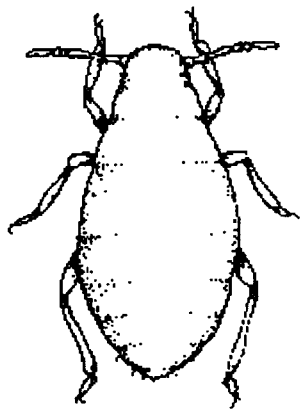
Figure 1. 3

Two generations of *Pemphigus spyrothecae*



1 mm

Fundatrix



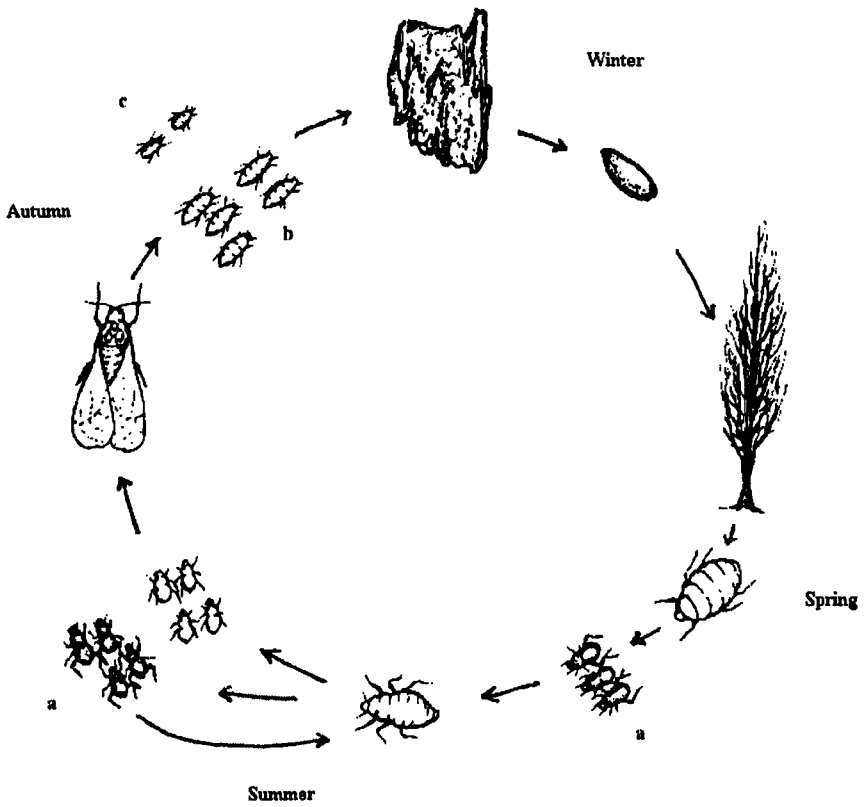
1 mm

Virginoparae

(adapted from Tótn, 1939)

Figure 1. 4

Life cycle of *Pemphigus spyrothecae*



KEY

a: Soldiers
b: Females
c: Males

Adapted from Foster & Northcott (1994)

Chapter 2

INTER-PLANT VARIATION AND THE EFFECTS ON APHID POPULATION DYNAMICS

2. 1 Introduction

Herbivores are seldom distributed randomly among host plants, but tend to aggregate on certain individuals of a population (Strong *et al*, 1984). Even when there appears to be no direct evidence of differences in host plant quality, herbivores may nevertheless non-randomly select a plant, e.g. butterflies on isolated plants, or at the edge of a clump of plants (MacKay & Singer, 1982; Courtney & Courtney, 1982). Recent studies show that differences in quality among individual plants may have important effects on the population dynamics of herbivores (Watt, 1990; Moran & Whitham, 1990; Memmott *et al*, 1995). Individual trees vary in their defence against insect attack, and to combat this variation, herbivores such as aphids and scale insects may become adapted to individual trees. For instance, scale insects transplanted onto a selection of pine trees showed a 16-fold difference in survival (Edmunds & Alstad, 1978) and nymphs of the aphid *Cinara cupressi* were significantly less likely to survive when transplanted onto cypress tress with low aphid infestation rates than onto those with high infestation rates (Memmott *et al*, 1995). The extent to which a particular host plant can influence the population densities of its associated herbivores depends on both ecological and evolutionary processes.

Host-plant heterogeneity may help explain how trees avoid complete defoliation despite the great reproductive potential of specialised insects. Trees with complex plant architecture show a great deal of variation in resistance to herbivory (e.g. Rohfritsch, 1981; Stoetzel & Tedders, 1981; Wool, 1984). Colonising aphids have been shown to exhibit a 75-fold difference in survival among individual trees growing in close proximity in the same grove (Whitham, 1983). Whitham also demonstrated enormous differences in survival of aphids among branches of the same tree, among leaves on the same branch, and even among positions on the same leaf (Whitham, 1978; 1980; 1981). He argued that an individual tree offers such a mosaic of varying susceptibilities to attack by aphids that somatic mutations could play a

major role in plant defence, generating a diversity of genotypes within a single plant (Whitham, 1981; Whitham & Slobodchikoff 1981).

I predicted that inter-plant differences exist among individual *Populus* x *canadensis* trees and that this would affect the population dynamics of *Pemphigus spyrothecae* aphids. I investigated a) architectural differences among trees; b) timing of budburst; c) stem mother emergence; d) variance in gall load and position on trees and e) variation in aphid performance among trees.

2. 2 Methods

The life cycle of *Pemphigus spyrothecae* Passerini, 1860 has been described by Dunn (1966) and Foster (1990). This species is not host alternating, but spends its whole life cycle on its primary host, the black poplar *Populus nigra* and its hybrids and varieties. Eggs laid the previous year in the bark on branches or on the trunk of the host tree, hatch in spring and produce parthenogenetic stem mothers (first generation). The wingless stem mothers are very mobile, and migrate out to the newly emerged shoots, where they select a petiole for gall formation. By midsummer up to several hundred offspring are parthenogenetically produced. The first instars grow into wingless adults (virginoparae, second generation), and these in turn produce both thick-legged and normal-legged first instars. The normal-legged instars grow into winged sexuparae (third generation), that, once the galls have opened in August, migrate to the trunk, give birth to sexuals (fourth generation), which mate and produce overwintering eggs in the bark of the tree.

The hybrid black poplar (*Populus* x *canadensis*) trees used in this study are located at Attenborough Gravel Pits, Nottingham, England, a Site of Special Scientific Interest (SSSI), jointly managed by Nottinghamshire Wildlife Trust and owners Butterley Aggregates Ltd. Mature trees were used, since juvenile trees are more resistant to galling and are avoided by migrants (Kearsley & Whitham, 1989). The trees grow close together along a stream near a disused car park, and hence are all subjected to similar climatic and environmental conditions. The trees were planted approximately 25 years ago.

A total of approximately 400 galled and un-galled shoots were unbiasedly collected prior to gall opening in early August from eight poplar trees, A-H. These were placed in a freezer at -20° C to prevent the final development and release of the alates (winged progeny). For convenience, I selected galls from the lower three metres of each tree, although this may have biased estimates of the within-tree variation in host defence. The size of individual leaves on the shoot was later measured using the greatest length and width in mm; leaf area was calculated by placing the leaf under a one cm^2 transparent grid, and summing the number of square cm units within the leaf

outlines. This method was also used to estimate the percentage leaf damage caused by feeding herbivores. Petiole length was measured in mm, and on galled petioles the position of the gall on the petiole was calculated as a percentage of the total length of the petiole, with 0% being at the shoot junction and 100% at the leaf junction. For the purpose of this study a shoot is defined as the product of a single season's growth from a single bud (Fay & Whitham 1990). The position of leaves on the shoot was recorded by ranking them from the apical leaf. Competitor density was measured as the number of galls per petiole. I classified as abortions those fundatrices that failed to initiate a successful gall, seen later as a kink in the petiole, or those that died before gall completion, forming an undeveloped empty gall. If two fundatrices move onto the same petiole, they may both initiate galls, but one or the other may leave before gall completion (Dunn, 1960). Individual galls were weighed using a Cahn electro-balance, and the number of aphids counted. Each gall was opened, the contents gently transferred with a paintbrush into a petri-dish containing 70% methcol, and the number of aphids and winged progeny counted under a Wild M-3 dissecting microscope.

The following April, 20 shoots were randomly checked on trees A-H each day to ascertain budburst and arrival of *Pemphigus* stem mothers. The buds were recorded as being 'closed', 'intermediate' or 'open' (Plate 2.1). The date of the first budburst on each tree was noted together with the earliest date all 20 shoots were open. The date of the first stem mother sightings was also recorded.

In order not to duplicate data, where appropriate, only measurements from singly-galled leaves were included in the analyses. All mean values are given ± 1 s.e., and sample sizes are given in the graphs. The data were checked for normality and where necessary non-parametric statistics were used for analyses.

2. 3 Results

Structural differences among trees were examined. Both mean number of leaves per shoot and mean shoot leaf area differed significantly among trees (Fig. 2.3.1). Trees G, H and D had most leaves per shoot (approximately seven) and greatest shoot area (between 90-100 cm^2), whilst A had on average only 5 leaves per shoot with a shoot leaf area of 40 cm^2. The mean leaf length and width were also significantly different among trees, with A and B having the smallest leaves and trees E-H the greatest mean size (Fig. 2.3.2), also seen for mean leaf area (Fig. 2.3.3). A and B had the smallest leaves, and trees G and H the largest. The mean petiole length ranged from 30mm on tree B to nearly 45mm on tree C (Fig. 2.3.4), and this was significantly different among poplar trees. I examined leaves from each tree for herbivore and/or weather damage. Damage to the leaf surface affects the level of photosynthesis and therefore plant growth. Fig. 2.3.5 shows the mean percentage intact leaf on trees A-H: tree H had the least damage and tree A the most, and these differed significantly among trees.

During April the trees were examined to determine how budburst coincided with the arrival of aphid stem mothers. The trees showed significant differences in both bud length and width prior to opening. Fig. 2.3.6 illustrates how the buds on trees A-H developed from closed to open shoots during April. The trees differed significantly in the rate at which shoots opened. Trees E and H showed first signs of budburst on the 7th of April followed by B and D on the 8th, then trees C, F and G on the 12th and lastly A on the 13th of April (Fig. 2.3.7). Tree G was first to have all 20 randomly selected shoots open on the 18th of April, followed a day later by C and H. F was fully open by the 20th, then tree E on the 22nd, and trees A, B and D finally opened on the 23rd of April. On trees B, D and E just over two weeks elapsed between first signs of budburst and all the randomly selected shoots being open. Trees H and A took 12 and ten days, respectively, F took eight days, and C and G only six days. Although a tree may show signs of early budburst, the shoots may not open any sooner than those with first shoots opening six days later, since all eight trees were fully open within five days of each other. In my study site, three of the four trees to show later budburst had all shoots opened first.

Stem mothers timed their emergence very closely to bud burst. All 20 randomly selected shoots on the trees were either already open or became so within three days, giving the stem mothers plenty of choice on which to initiate galls. Stem mothers were first seen on tree F on the 18th of April, the next day on tree G, and on the remaining trees a day later. Thus all trees had *Pemphigus* stem mothers within two days (Fig. 2.3.7). Because only 20 shoots were recorded each day, it is possible that stem mothers may have emerged on the same day on all trees.

Since the trees showed significant intraspecific variation in terms of shoot size and structure and leaf size, I hypothesised that *Pemphigus* aphids would exhibit variation in performance, gall location and numbers. I examined 216 shoots at random from eight poplar trees and found no significant differences in the number of galls supported on each shoot. Tree C had, on average, the highest number of galls per shoot, whereas trees G and H had the fewest (Fig. 2.3.8). Likewise, there were no differences in the average number of galls on each leaf. Here tree B had the lowest mean number and trees E and H had the highest number of galls per leaf (Fig. 2.3.8). Of the 492 galled leaves examined on trees A-H, the percentage of doubly-galled leaves ranged from 8.5% on tree B to 24% on tree D.

I next examined intraspecific variation in gall position on the shoot and again found no significant differences among the eight trees (ANOVA $F_{7,288} = 1.5$, n.s.). There were, however, significant differences in the positioning of the gall along the petiole (ANOVA: distance of gall to leaf $F_{7,300} = 2.96$, $p < 0.01$; distance of gall to base of petiole $F_{7,300} = 4.50$, $p << 0.001$). Galls on trees A and F were, on average, closer to the leaf junction than tree D (Fig. 2.3.4). The only significant intraspecific variation in gall numbers and location was the positioning of the gall on the petiole. Interestingly, on trees A and B with, on average, the shortest petiole, the galls are located closer to the leaf junction than on the other poplar trees (Fig. 2.3.4).

Both the number of aphids and the number of alates per gall differed significantly among trees. The trees furthest from the old entrance to the reserve, trees F-H, had, on average, more *Pemphigus* wingless adults than those closest to the entrance (A and B), but tree D had the highest number of winged progeny than any other tree (Fig. 2.3.9).

Likewise, trees F-H had the heaviest galls, and tree B the lightest galls (Fig. 2.3.10), and this differed significantly among trees. Lastly, gall success differed significantly among the sample trees (Fig. 2.3.11). Tree B had the highest rate of failure, and tree G the lowest at only 5%. Of the 451 galls examined, 85.8% became mature galls, giving an overall failure rate across trees of 14.2%.

In order to test the significance of association between the variables examined above, I have ranked the variates and calculated a coefficient of rank correlation, Kendall coefficient of concordance W (Table 2.3.1). This indicates that the rankings are concordant, and that on an overall scale I can conclude that trees G, H and F provide a better quality habitat for *Pemphigus* aphids than trees A and B.

2. 4 Discussion

The effect of host plant variation on herbivore populations is a popular research topic: studies have shown plant quality to be an important factor in limiting insect population density by affecting development, mortality and growth rates (e.g. Kidd *et al*, 1990; Whitham, 1983; Whitham *et al*, 1984). Variation in host plants can be manifested in bud burst phenology, plant chemistry or morphological characteristics (e.g. Zucker, 1982; Akimoto, 1990), and also in the genetic variation in resistance to herbivory (e.g. Fritz & Nobel, 1989; Memmott, Day & Godfray, 1995; Whitham, 1983). As expected, inter-plant variation was confirmed in this study: the eight *Populus* x *canadensis* trees differed significantly in all eight variables chosen to represent morphological characteristics.

The difference in a host plants response to aphid attack can be quite extreme, and whether trees will be successfully colonised by aphids may depend on physiological, nutritional, ecological or genetic factors on both the part of the aphid and the trees (Wool, 1984). Colonising aphids have been shown to exhibit a 75-fold difference in survival among individual trees growing near one another in the same grove (Whitham, 1983), and while all trees are not equally susceptible to attack (Grigarick & Lange, 1962; Whitham, 1989), several authors report cases where some trees were heavily infested with insect galls, whilst others in close proximity never developed a single gall (Rohfritsch, 1981; Stoetzel & Tedders, 1981; Wool, 1984; Gilbert *et al*, 1994). Although the poplars used in this study all had aphid galls, there were significant differences in aphid performance. For instance, tree B had not only the highest gall failure rate, but also the fewest number of aphids per gall. One explanation could be the small mean leaf size found on tree B (see Chapter 3 on within plant variation). Aphids feed on plant nutrients, and since smaller leaves produce less photoassimilates than larger leaves, small leaves could be considered to be a poorer habitat. A reduction in reproductive success on poor quality host plants is not uncommon; genetically identical vetch aphids (*Megoura viciae*) reared on poor quality plants weighed significantly less, had on average fewer ovarioles and proportionately fewer offspring with a high ovariole number than those on high quality plants (Walters, Brough & Dixon, 1988).

Genetic differences among clones of arroyo willow, grown under the same environmental conditions, had a significant effect on densities of four sawfly species (Fritz *et al*, 1986; Fritz & Price, 1988). Sawflies are known to use certain plant characteristics as oviposition cues; not only did willow clones differ significantly in morphological traits such as mean shoot length (Fritz & Price, 1988), but willows with long leaves were found to be more susceptible to galling than those with short leaves (Fritz & Nobel, 1989). Although *Pemphigus spyrothecae* aphids showed no among-tree differences in gall location, they achieved a reproductive advantage on trees with greater shoot and leaf size. In other insect systems the size of host shoots can also have a negative effect on population densities. For instance, thicker intact *Phragmites australis* shoots led to higher mortality rates in the female gall midge *Giraudiella inclusa*. Reproductive success was greater on thin shoots, stressed by water or nutrient deficiency, or on damaged thick shoots, stressed by stem-miners (Tscharntke, 1988). Although stem-miners enjoyed a reproductive advantage on damaged or stressed shoots, a high degree of leaf damage on poplar trees may affect the rate at which *Pemphigus spyrothecae* alates are produced (both trees A and E had high rate of leaf damage and a low mean number of alates per gall).

For many herbivorous insects that attack unfolding leaves in spring, the timing of budburst of their host plants provides heterogeneous and unpredictable environments, because budburst varies within and among host individuals and within years (Dixon, 1976; Crawley & Akhterauzzaman, 1988; Komatsu & Akimoto, 1995; Mopper & Simberloff, 1995; Akimoto, 1990). During a period of several years, particular individual oak trees were consistently first to budburst and the same ones last to flush (Crawley & Akhteruzzaman, 1988). Leaf fall was found to follow a similar pattern. Some herbivores appear to have overcome the difference in budburst between the earliest and latest trees. The leaf miner *Eriocrania subpurpurella* is more suited to the early flushing trees, while others such as the aphid *Phylloxera quercus* were more numerous on late flushing trees. Although the poplar trees in this study showed a significant difference in timing of budburst in the study year, whether this difference is consistent over years remains unknown. Crawley & Akhteruzzaman (1988) suggested that the wide range of budburst dates in *Quercus robur* ensures that at any

given time there is a considerable range of leaf ages available to ovipositing female insects.

The importance of budburst synchrony on insect performance has been reported on many occasions (e.g. Akimoto, 1990; Hunter, 1992; Komatsu & Akimoto, 1995; Kerslake & Hartley, 1997). Mean egg-hatching time of the galling aphid *Kaltenbachiella japonica* was positively correlated with mean budburst time on individual trees, suggesting that aphids are genetically adapted to the budburst phenologies of their host tree (Komatsu & Akimoto, 1995). *Pemphigus spyrothecae* is non-migratory like *Kaltenbachiella japonica*, and although *P. spyrothecae* stem mothers timed their emergence to budburst (shoots examined were already open or became so within three days of fundatrix emergence), all trees had stem mothers within two days. Any tree-specific microadaptation would need to be examined over a period of years. Edmunds & Alstad (1978) theorised that adaptation of herbivores to individual host plants is likely if plants are long-lived and have traits different among individuals, and if herbivores are so sedentary that there is little gene flow between populations on individual plants.

Since only a small portion of the variance in insect abundance is attributable to budburst (c.f. Crawley & Akhteruzzaman, 1988), there obviously must be other differences among phenotypes of trees, not easily recognisable, but of great importance to the aphids. Sex pheromones from ovipositing sawflies may encourage oviposition by other individuals and microclimatic differences around the host plants may affect oviposition behaviour (Fritz *et al*, 1987). Past aphid infestation may weaken the host and make it more susceptible to future attack or alternatively, induce a resistance to future attack (Memmott, Day & Godfrey, 1995). Lewis (1987) reported that high densities of *Schizolachnus pineti* on Scots pine reduced the foliage quality for later generations of aphids. Other important differences in plant phenology could be crown volume and degree of leaf retention: *Populus* is colonised by migrants of *Pemphigus betae* in autumn, and both crown volume and leaf retention correlate with stem mother survivorship the following spring (Moran & Whitham, 1990). Visual leaf-based cues followed by leaf chemical characteristics once alighted may help the aphid to colonise better quality hosts.

Plants may also vary in the level of biochemical defence against herbivory. Aphid nymphal growth rates are positively correlated to variation in total phloem phenolics and certain amino acids in conifers (Kidd *et al*, 1990). There are also significant seasonal and among tree differences in certain individual flavonoid glycosides. Kidd (1994) studied the large pine aphid, *Cinara pinea,* on Scots pine (*Pinus sylvestris*) and found that taxifolin was correlated to aphid performance among trees. Plant toxins are also known in some cases to be beneficial to herbivores. Taper & Case (1987) reported a positive correlation between leaf-tannin levels and cynipid diversity. They hypothesise that tannins aid in the defence of cynipid larvae either from hyperparasites, from leaf herbivores, from fungal pathogens or a combination of these. Phenolics may account for some variation in aphid performance among the *Populus* trees in this study.

Lastly, Larson & Whitham (1997) put forward the 'sink competition hypothesis' to explain the distribution of phloem parasites among host plants. Galling aphids feed on the photoassimilates and nutrients moving through the cottonwood from sites of production or storage (sources) to sites of active use or growth (sinks) by tapping into the host vascular system and creating a sink (Larson & Whitham, 1991). Resistant trees had almost twice as many buds per unit of stem volume as susceptible trees, so stem mothers forming galls on resistant trees face a higher ratio of sinks to sources and potentially stronger competition among sinks during spring allocation of stored reserves than on susceptible trees, i.e. resistant cottonwoods have more competing sinks in early spring when an increased allocation to the galled shoot is required to produce a gall. By reducing the number of competing sinks (i.e. buds) on branches of resistant tree genotypes to imitate bud densities of susceptible genotypes, aphid survival significantly increased as predicted (Larson & Whitham, 1997). Because the architecture of a plant is reflected in its sink-source relationships (Waller, 1986), the architectural form of a plant can be expected to be a significant aspect of host quality for gallers.

To summarise, I have determined that individual *Populus* x *canadensis* trees, in close proximity, vary considerably in architectural and budburst phenology. Some trees appear to support larger *Pemphigus spyrothecae* populations than others. As described

above, much research has shown that variability in plant architecture among natural plant populations is common and widespread, and has a strong impact on aphid fitness. It is extremely difficult to pinpoint any single causal link, but is likely to be due to a number of factors such as genetics, age, environmental stress, herbivore damage and biochemistry.

Table 2. 3. 1

TREE / VARIABLE	A	B	C	D	E	F	G	H
No leaves/shoot	8	4	7	3	6	5	1	2
Shoot leaf area	8	6	7	3	5	4	1	2
Leaf length	8	7	5	6	3	4	2	1
Leaf width	7	8	3	6	5	4	2	1
Leaf area	8	7	3	6	4	5	2	1
Petiole length	8	7	2	5	6	1	4	3
% Intact leaf	7	5	6	2	8	4	3	1
Galls/shoot	7	3	8	6	3	5	1	3
Galls/leaf	2	1	5	6	8	4	3	7
Gall weight	4	8	7	6	5	2	1	3
Aphids/gall	5	8	6	3	7	4	1	2
Alates/gall	6.5	6.5	5	2	8	4	1	3
Budburst	6	6	2	6	4.5	4.5	2	2
% Failures	3	8	6	7	5	2	1	4
MEAN **± 1 s.e.**	**6.25** **0.54**	**6.04** **0.57**	**5.14** **0.52**	**4.79** **0.47**	**5.54** **0.46**	**3.75** **0.33**	**1.79** **0.26**	**2.50** **0.26**

The mean values of each variable have been ranked across trees, with rank 1 = the largest value except **Galls/shoot, Galls/leaf** and **% Failures**, where rank 1 = the smallest value. The smallest value for **Budburst** refers to the date when all the buds were open closest to the arrival of the stem mother. Kendall's Coefficient of Concordance W was calculated and transformed using X^2=k(N-1)W, where k = number of variables (13), N = number of trees (8) and W=Coefficient of Concordance. This is distributed as $X^2_7 = 44.92$, $p<0.001$.

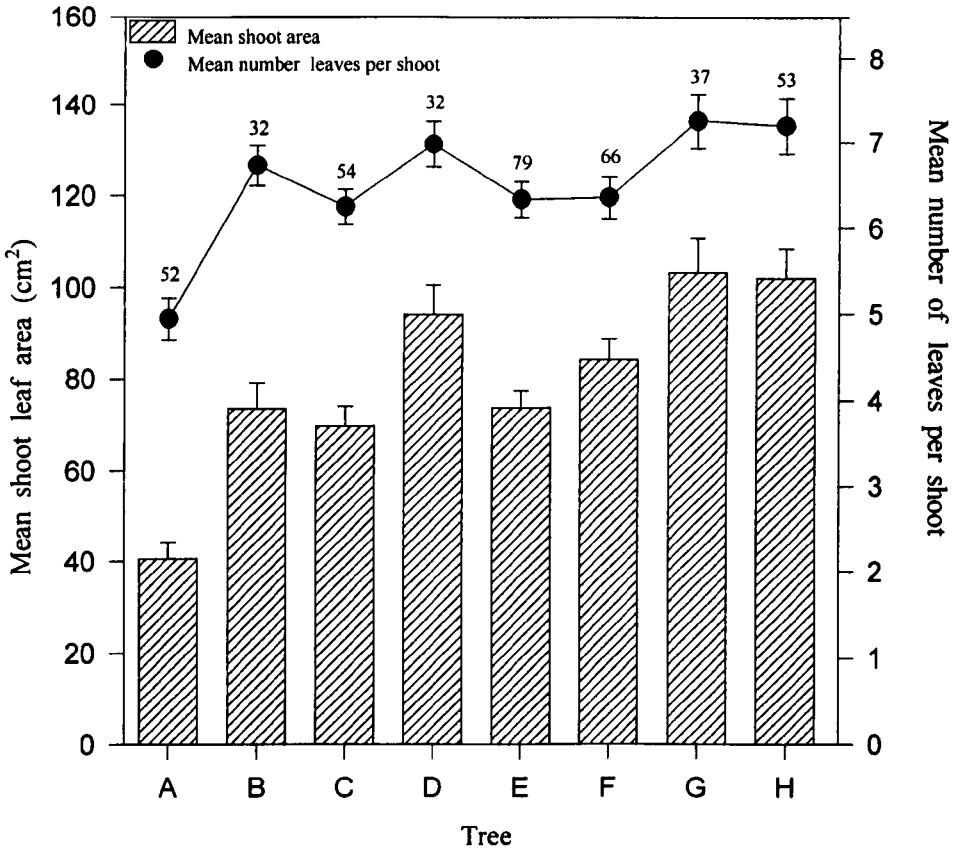
Plate 2. 1

Closed Intermediate Open

At budburst the *Populus* x *canadensis* shoots were recorded as being either 'closed', 'intermediate' or 'open'.

Figure 2. 3. 1

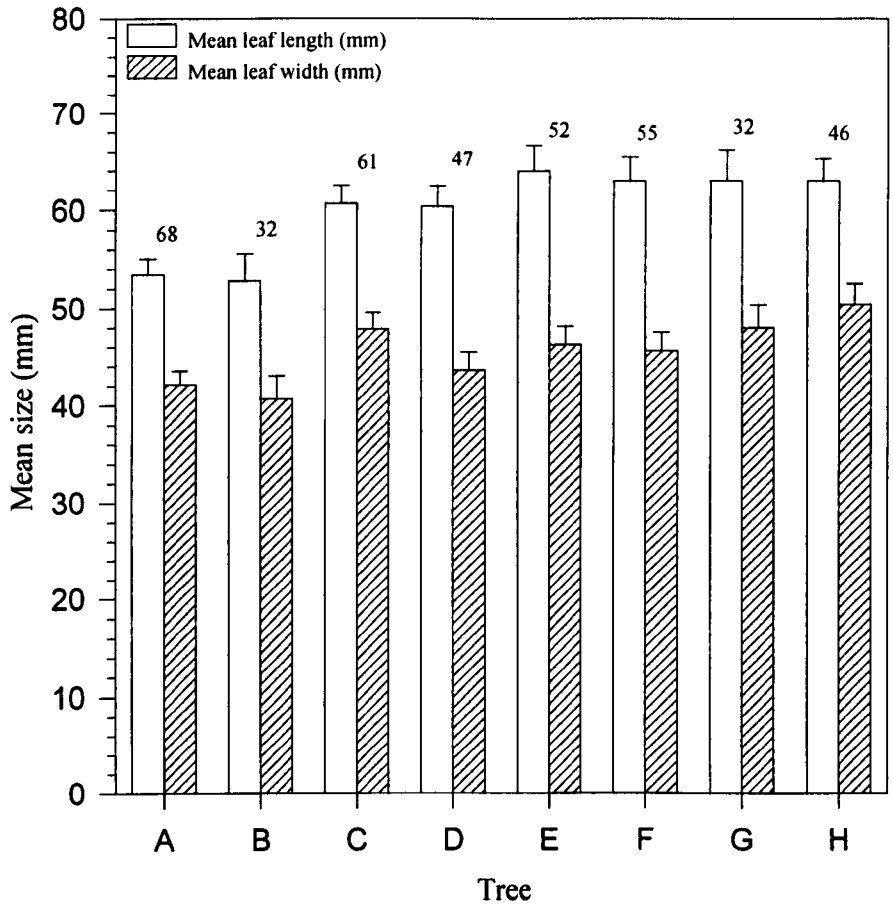
Differences in shoot size among trees



Both the number of leaves per shoot and shoot leaf area differed significantly among the eight poplar trees (ANOVA: number of leaves per shoot $F_{7,397} = 7.77$, $p << 0.001$; shoot area $F_{7,397} = 15.39$, $p << 0.001$).

Figure 2. 3. 2

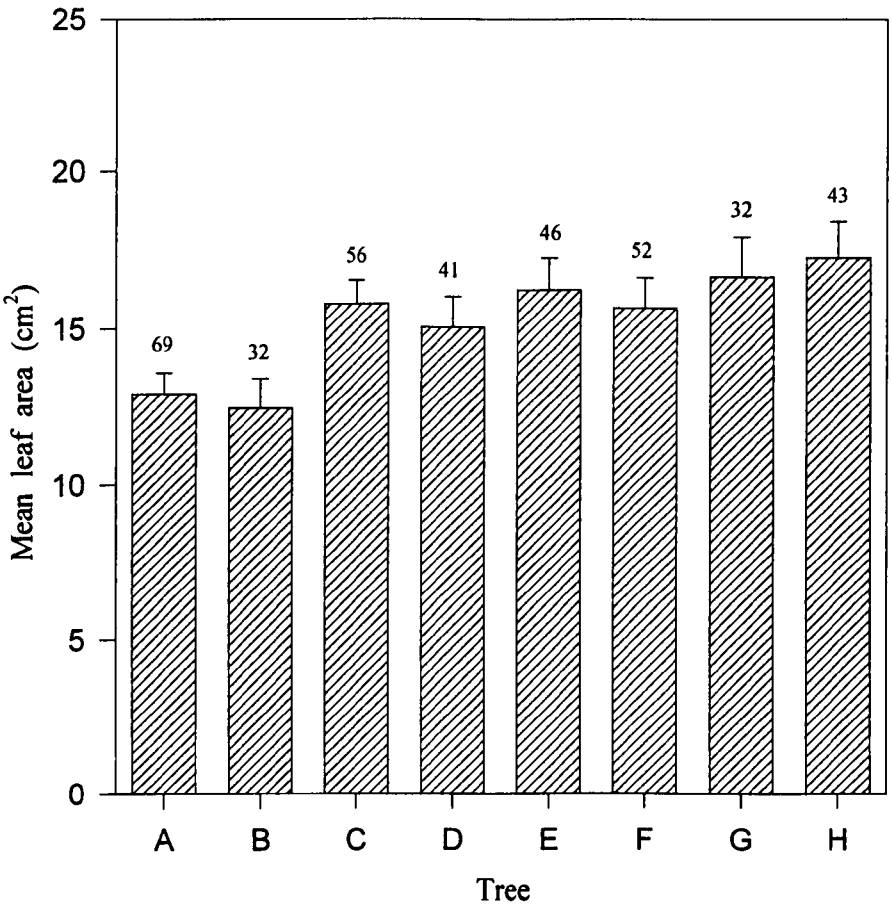
Differences in leaf length and width among poplar trees



There were significant differences in both leaf width and length among the poplar trees (ANOVA: leaf width $F_{7,373} = 3.75$, $p < 0.01$; leaf length $F_{7,373} = 2.77$, $p < 0.01$).

Figure 2. 3. 3

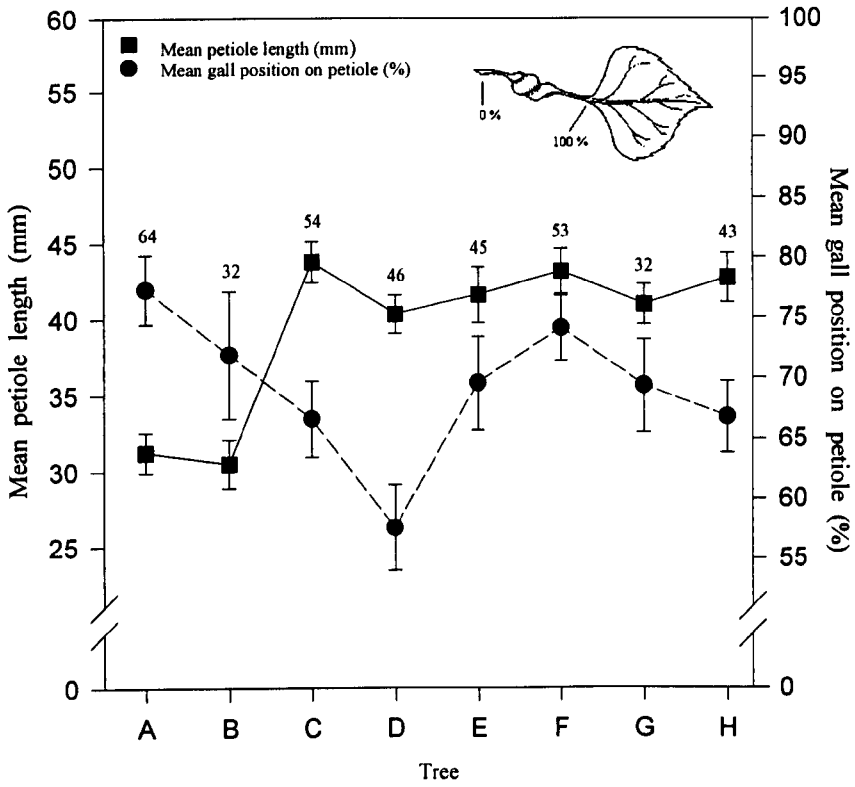
Among tree differences in leaf area



There were significant differences in leaf area among *Populus* x *canadensis* trees (ANOVA: $F_{7,370} = 3.24$, $p < 0.01$).

Figure 2. 3. 4

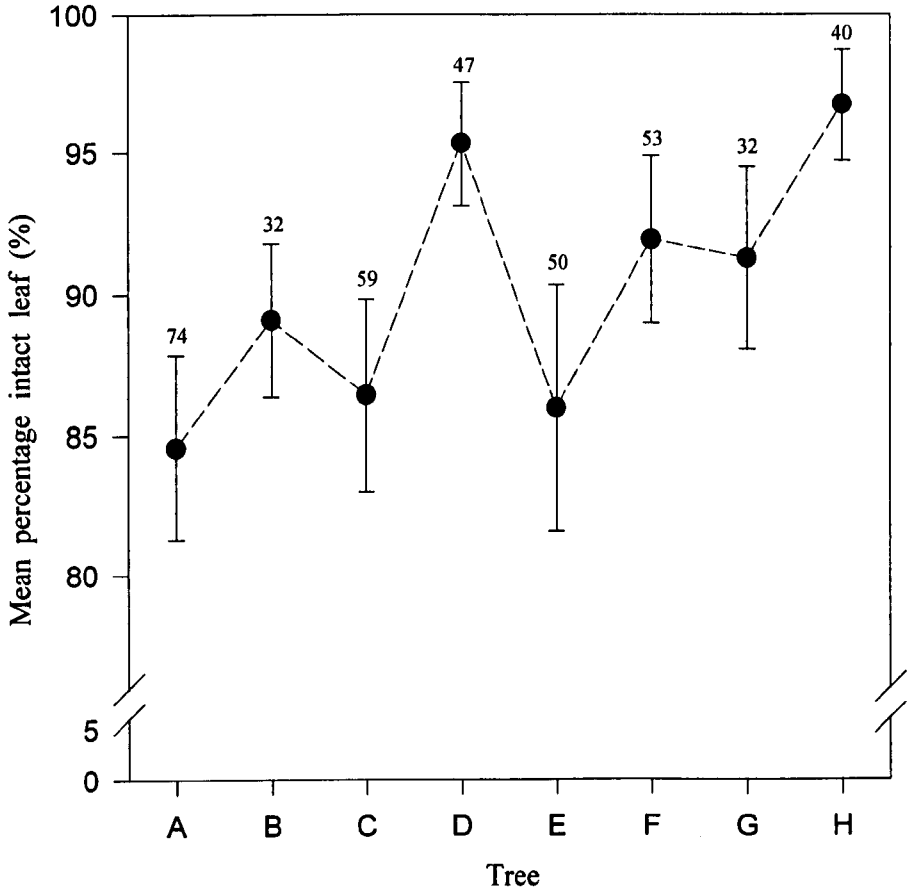
Differences in poplar petiole length and *Pemphigus* gall position on petioles



The average length of poplar petioles differed significantly among trees (ANOVA: $F_{7,368} = 12.84$, $p << 0.001$), and there were significant differences in gall position on singly galled petioles among trees (Kruskal Wallis $H_{7, 368} = 25.04$, $p < 0.001$).

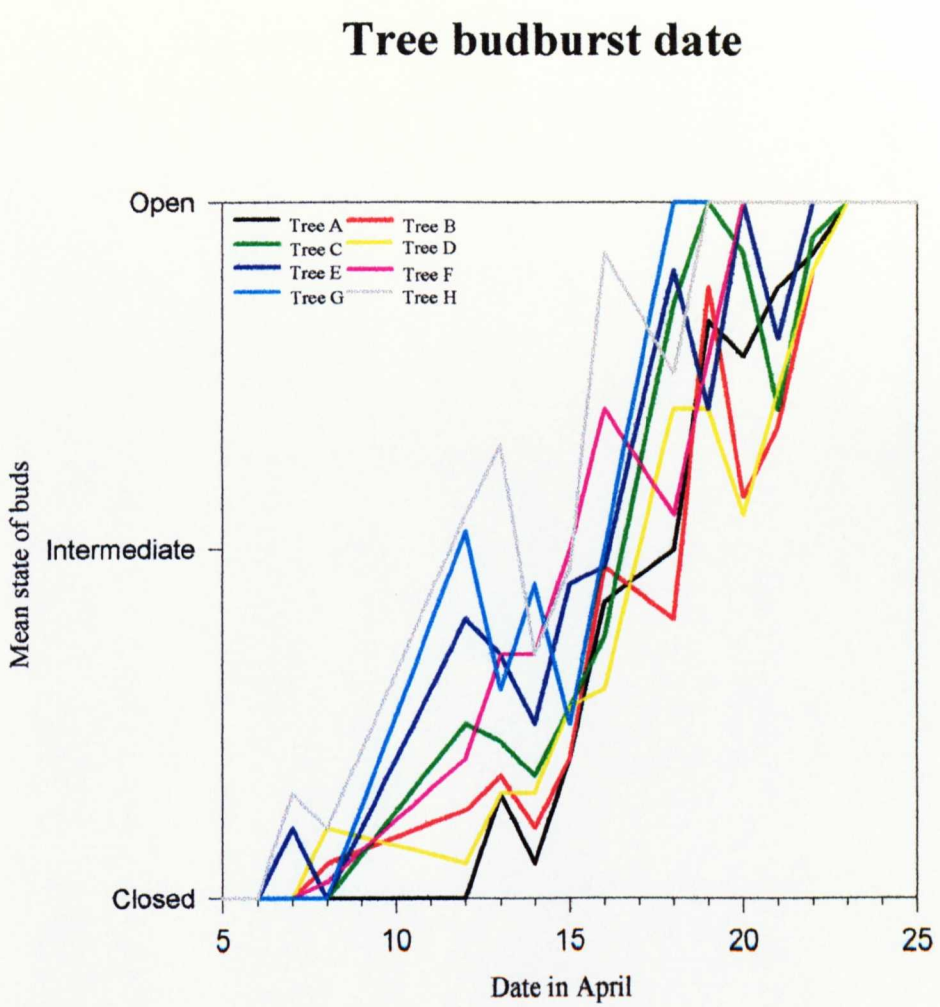
Figure 2. 3. 5

Percent intact leaves among poplar trees



There were significant among tree differences in the percentage intact leaf surface (Kruskal Wallis $H_{7,387} = 33.61$, $p < 0.001$).

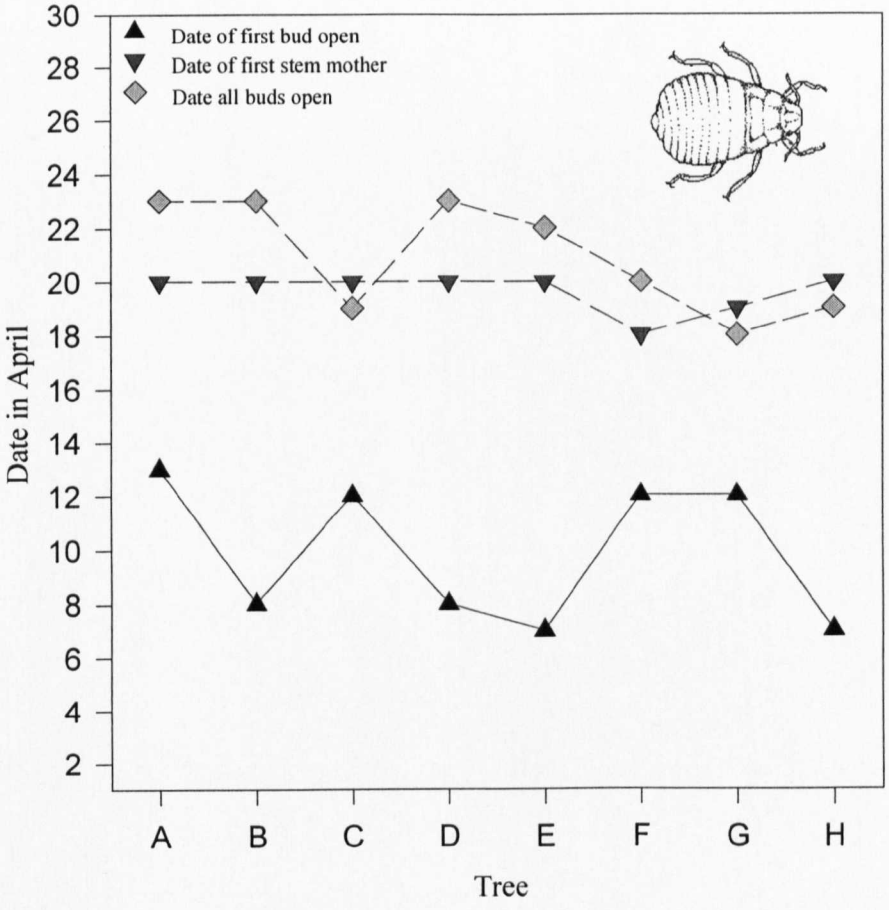
Figure 2. 3. 6



Buds were collected unbiasedly from the poplar trees during April. These were coded as 'closed', 'intermediate' or 'open' (Plate 2.1). For clarity, only eight trees are illustrated in this figure. By the 20th of April half of the trees had all collected buds fully open. Prior to budburst there were significant differences in both bud width and bud length among trees (ANOVA: bud width $F_{12,759} = 15.14$, $p << 0.001$; bud length $F_{12,759} = 19.52$, $p << 0.001$).

Figure 2. 3. 7

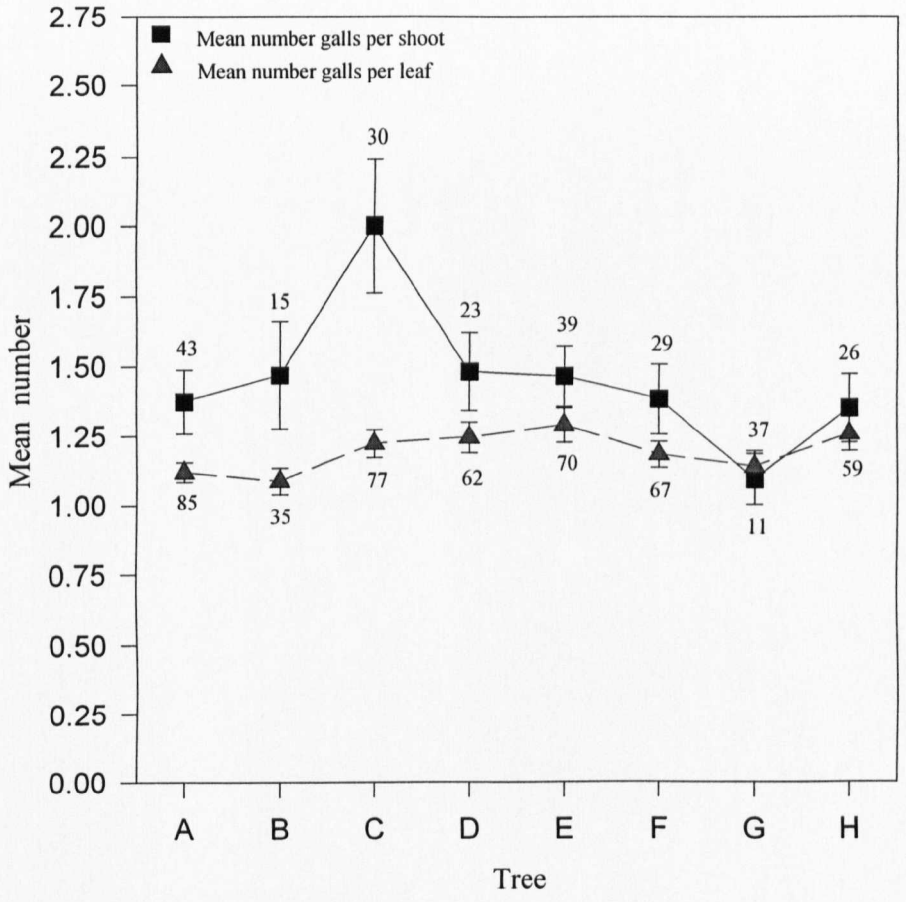
Budburst date and *Pemphigus* stem mother arrival on poplar trees



This figure illustrates the date on which each tree had one of the collected buds open, the date that all collected buds were open, and the first signs of *Pemphigus* stem mothers. There was a significant interaction between trees and date on budburst (2-way ANOVA: $F_{133, 3199} = 3.06$, $p << 0.001$).

Figure 2. 3. 8

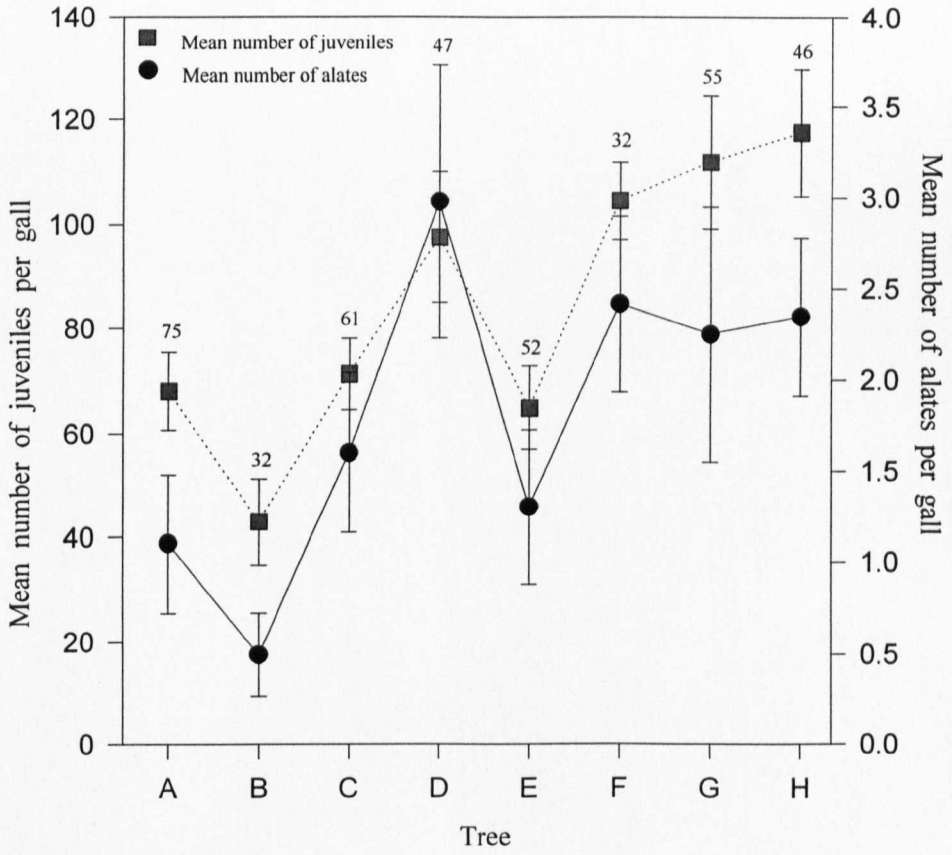
Among tree differences in the number of *Pemphigus* galls per shoot and galls per leaf



There were no significant differences in the number *P. spyrothecae* galls per shoot (Kruskal Wallis $H_{7,216} = 7.43$, n.s.) or the number of galls per leaf (Kruskal Wallis $H_{7,492} = 4.90$, n.s.) among poplar trees.

Figure 2. 3. 9

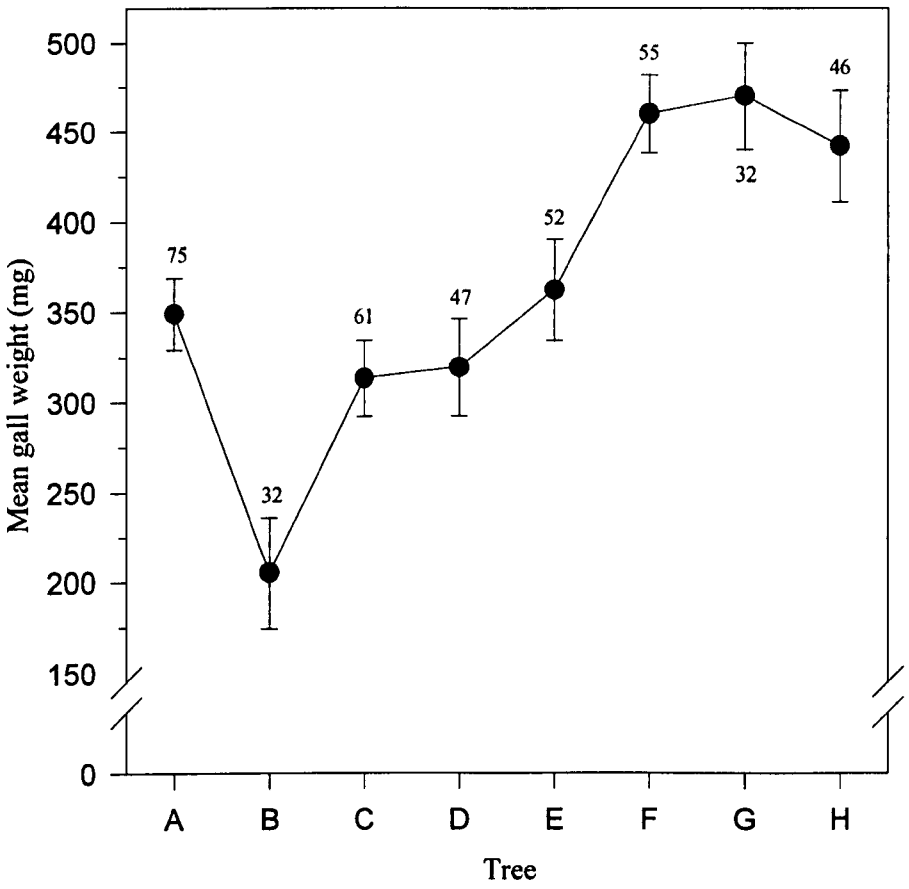
Number of *Pemphigus* juvenile and alates per gall among poplar trees



There were significant differences among trees in both the average number of juveniles and alates per gall (number of juveniles per gall: Kruskal Wallis $H_{7,399}$ = 47.73, $p < 0.001$; number of alates per gall: $H_{7,399} = 18.25$, $p < 0.05$).

Figure 2. 3. 10

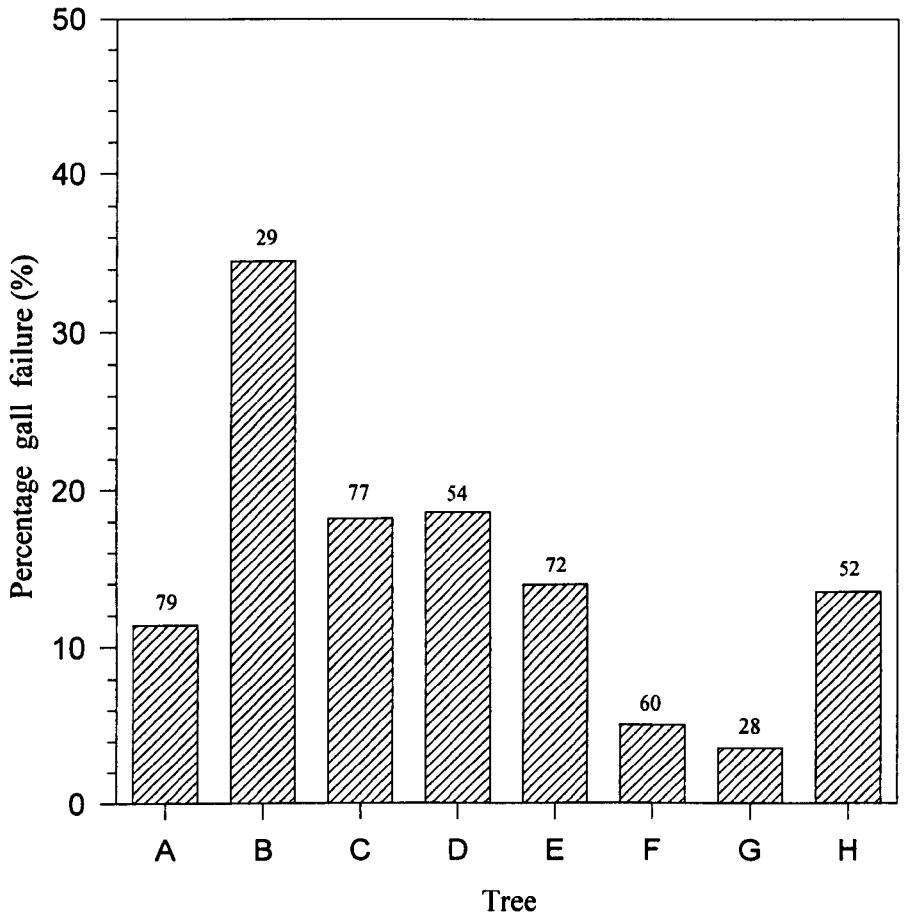
Among tree differences in *Pemphigus* gall weights



There were significant among tree differences in average gall weight (ANOVA: $F_{7,399} = 9.64$, $p << 0.001$).

Figure 2. 3. 11

Percentage failed *Pemphigus* galls among poplar trees



The percentage of failed *Pemphigus* galls differed among trees ($\chi^2_7 = 18.94$, $p < 0.01$, $n = 451$).

Chapter 3

WITHIN-PLANT HETEROGENEITY AND THE EFFECTS ON APHID POPULATION DYNAMICS

3. 1 Introduction

A critical event in the life history of gall-forming insects is the choice of galling site (Weiss *et al*, 1988; Whitham, 1978). Within just a few days of selecting a galling site, the reproductive outcome of a female galling aphid is determined by the choice of leaf. Most aphids feed from phloem tissues by siphoning phloem sap as it flows under positive pressure from the leaves on the host plant. Therefore, the quantity and quality of this sap should determine on which leaves the aphids will settle to feed.

The within-plant distribution of galls has been examined in many studies. For instance, adjacent *Populus angustifolia* branches and also branches at various heights showed very different patterns of *Pemphigus betae* gall distribution (Whitham, 1983): there was a 42-fold difference in aphid preference for one branch over another. In another example, *Adelges cooleyi* were seldom found in the top-most whorls of shoots of Engelmann spruce: galling in these top whorls resulted in a mortality rate of nearly 100%, whereas gallers colonising the bottom whorls had a survival rate of at least 60% (Fay & Whitham, 1990).

Some authors have demonstrated that leaf area is correlated with greater survival and fecundity of gall-formers and leaf miners (e.g. Whitham, 1978; Tuomi *et al*, 1981; Bultman & Faeth, 1986). Other studies show that leaf quality rather than size is important for survival (Schultz, 1983; Sitch *et al*, 1988). Net primary productivity is often related to photosynthetic surface area, thus the size of the leaf should correlate with the quantity/quality of resources translocated via the leaf vascular tissues. Previous studies have shown that gall-formers become integrated into the host plant sink-source transport system (e.g. Whitham & Larson, 1991). This happens early in the season, and gallers establish a long-term physiological relationship with their host plant. Therefore the position of the gall on the leaf may be important to *Pemphigus*

stem mothers, because the gall position determines the effectiveness with which the gall acts as a resource sink by drawing resources from neighbouring leaves (Larson & Whitham, 1991). Galls can also affect photosynthetic rates of surrounding plant parts, probably by changing the nutrient status of leaves (due to the sink-competition between young leaves and growing galls) thus reducing both photosynthesis in galled leaves and the neighbouring leaves (Larson, 1998).

I examined within-plant habitat selection in *Pemphigus spyrothecae* aphids galling on *Populus* x *canadensis* shoots. Stem mothers, when selecting a leaf for gall initiation, spend a lot of time (up to 51 minutes) visiting a few of the leaves and petioles on the shoot, though many leaves will not be visited at all. Probing was observed lasting up to 3.5 hours, with some aphids dying before showing any signs of gall formation, even though they survived in the laboratory for up to 5 days (Beyer, 1993). I predicted that a) aphids would obtain a greater fitness on larger shoots and would therefore most frequently be located on these shoots and b) that sharing a shoot with other galls would result in a loss of fitness. I further predicted that c) aphid galls would most likely be placed on the petioles of large leaves resulting in an increase in fitness compared to galls on smaller leaves, and d) that galls would be positioned on the petiole in order to maximise nutrient uptake from both the galled leaf and the surrounding leaves.

3. 2 Methods

The life cycle of *Pemphigus spyrothecae* Passerini, 1860 has been described by Dunn (1966) and Foster (1990). This species is not host alternating, but spends its whole life cycle on its primary host, the black poplar *Populus nigra* and its hybrids and varieties. Eggs laid the previous year in the bark on branches or on the trunk of the host tree, hatch in spring and produce parthenogenetic stem mothers (first generation). The wingless stem mothers are very mobile, and migrate out to the newly emerged shoots, where they select a petiole for gall formation. By midsummer up to several hundred offspring are parthenogenetically produced. The first instars grow into wingless adults (virginoparae, second generation), and these in turn produce both thick-legged and normal-legged first instars. The normal-legged instars grow into winged sexuparae (third generation), that, once the galls have opened in August, migrate to the trunk, give birth to sexuals (fourth generation), which mate and produce overwintering eggs in the bark of the tree.

The hybrid black poplar (*Populus* x *canadensis*) trees used in this study are located at Attenborough Gravel Pits, Nottingham, England, a Site of Special Scientific Interest (SSSI), jointly managed by Nottinghamshire Wildlife Trust and owners Butterley Aggregates Ltd. Mature trees were used, as juvenile trees are resistant to galling and are avoided by migrants (Kearsley & Whitham, 1989). The trees grow close together along a stream near a disused car park, and hence are all subjected to similar climatic and environmental conditions. The trees were planted approximately 25 years ago.

In order to examine habitat selection I collected 500 galled and 100 ungalled shoots from (the lower 3 metres for ease) the poplar trees. The shoots were unbiasedly collected prior to gall opening in early August, and placed in a freezer at -20° C to prevent the final development and release of the alates (winged progeny). The size of individual leaves on the shoot was later measured using the greatest length and width in mm; leaf area was calculated by placing the leaf under a one square cm transparent grid, and summing the number of square cm units within the leaf outlines. On galled petioles, the distance between gall and leaf base was measured in mm. The percentage

position on the petiole was calculated; 0% being at the base of the petiole and 100% being at the top of the petiole at the leaf junction. For the purpose of this study a shoot was defined as the product of a single season's growth from a single bud (Fay & Whitham, 1990). The position of leaves on the shoot was recorded by ranking them from the apical leaf. I classified as abortions those fundatrices that failed to initiate a successful gall, seen later as a kink in the petiole, or those that died before gall completion, forming an undeveloped empty gall. If two fundatrices move onto the same petiole, they may both initiate galls, but often one or the other may leave before gall completion (Dunn, 1960). Individual galls were weighed using a Cahn electro-balance. Since galls are initiated within the same period in spring, the data represents a cohort. Counting the number of progeny per gall gives an indication of the individual fitness of the stem mother. Each gall was opened and the offspring were gently transferred with a paintbrush into a small petri-dish containing 70% methcol. The number of nymphs and winged adults were counted under a Wild M3 dissecting microscope. To examine variation within trees, all variables were standardised among trees by subtracting the mean and dividing by the standard deviation for that tree.

Net primary productivity is related to photosynthetic surface area, therefore the size of a leaf can be used as a measure of the quantity and quality of essential resources translocated via the leaf vascular tissue; for instance, the amount of nitrogen in a leaf can be a function of its size (Gorden, 1971). To examine the effects of photosynthetic leaf area on *Pemphigus spyrothecae* fitness, I designed an experiment aimed at manipulating leaf area availability to the developing galls. A set of galls on trees D, E and F were selected which matched each other as close as possible in size, leaf size, and position on the petiole. Three different treatments were performed in spring on leaves of petioles carrying galls (Plate 3.2.1). The first manipulation involved total removal of the leaf 'removal', the second group had the leaf lamina removed 'lamina', and the final treatment involved cuts in the leaf lamina 'cut', but no actual damage was done to the leaf veins. Together with an undamaged control group, all treatmented galls were caged with sticky tape to exclude the effects of predation.

In order to view the relationships between fitness components, diagrams were constructed to illustrate the pattern of influences among components. They are based on the results of multiple regressions using only exploratory data analyses presented *a posteriori*, without adding or dropping variables to maximise the proportion of explained variation, which can cause misleading inferences about causal pathways (Petraitis *et al*, 1996). Path analysis is useful as it may suggest mechanisms of phenotypic selection (Kingsolver & Schemske, 1991). However, this method must be used with caution and preferably presented *a priori*, i.e. used to formulate a model which is later tested by collecting new data (Petraitis *et al*, 1996). Partial regression coefficients tend to reflect the original units in which the variables were measured, so I used them in standardised form, the beta weights: they give the change in standard deviation units in the dependent variable produced by a change of one standard deviation in the independent variable concerned (Kinnear & Gray, 1994).

Multicollinearity is a common problem interfering with proper interpretation of multiple regression by increasing the standard errors of path coefficients. Large standard errors of path coefficients indicate that the estimated strengths of effects between variables may vary considerably among samples resulting in less accuracy. Collinearity also affects the absolute magnitude of the partial regression coefficients, again reducing precision (for full reviews see: Myers, 1990; Petraitis *et al*, 1996; Kingsolver & Schemske, 1991). In this study I check for the absence of multicollinearity by confirming that the variance inflation factors (VIFs) are below 4 and that all R^2_is (the fraction of all variance in one X variable that can be predicted from the other X variables) are below 0.75. (InStat GraphPad Software, 1998)

Sample sizes and standard errors are included where possible in graphs showing mean values. The data were checked for normality, and where necessary, non-parametric statistics were used for analyses.

3. 3 Results

44.5% of all *Populus* x *canadensis* shoots (n = 407) contained either 7 or 8 leaves, and most *Pemphigus spyrothecae* galls were found on shoots of this size. Reproductive success was correlated with the number of leaves per shoot: for shoots with only one gall, heavier galls (Fig. 3.3.1) and more aphids (Fig. 3.3.2) were produced on shoots with a greater number of leaves. On average, an extra leaf on the shoot increases gall weight by 33.4 mg and the number of aphids by 9.4. Shoots without galls tended to have fewer leaves than those with one or more galls (Fig. 3.3.3). More galls were found on shoots with a larger shoot leaf area (Fig. 3.3.4). When several galls shared the same shoot, on average, the weight (Fig. 3.3.5) and reproductive success (Fig. 3.3.6) of individual galls decreased, although gall failure fluctuated (Fig. 3.3.7); each additional gall decreased mean gall weight by 27.6 mg and aphid numbers by 9.1.

Leaves are pre-formed in the leaf primordia and are available when stem mothers colonise immature leaves at bud burst. The basal leaves are unfolded first, so the leaves at the top half of the shoot tend to be a few days younger, but these are not necessarily the largest (pers.obs.). Dividing leaves on shoots into apical, upper middle, lower middle and basal groups, the average size of the middle leaves of shoots is larger than both the apical and basal leaves (Fig. 3.3.8). In the study year, over 50% of stem mothers initiated their galls in the upper middle part of the shoot (Fig. 3.3.8). The most frequently occurring leaves are between 10-20 cm^2 in surface area, with fewer than 10% of individual leaves having a surface area greater than 25 cm^2. On shoots with only a single gall, gall weight differed significantly but only slightly with position, with the heaviest galls on the upper-middle leaves (Fig. 3.3.9); there were no differences detected in reproductive success with gall position on the shoot (Fig. 3.3.10), although the pattern of mean values mirrors that of gall weight. Galls occurring singly on a shoot were heavier and supported more aphids as the shoot size increased (Figs. 3.3.11 and 3.3.12), and the relationships look curvilinear upwards. However, aborted or failed galls appeared equally on all shoot-size categories (Fig. 3.3.13). On leaves with only a single gall, although there was no difference in the rate of gall failure (Fig. 3.3.14), larger individual leaves supported heavier galls (Fig. 3.3.15) and resulted in greater reproductive success (Fig. 3.3.16).

Since nutrients are translocated into or out of a leaf, Whitham (1978) suggested that vascular feeders should prefer the base of the leaf blade at the junction of the petiole for gall formation. I predicted therefore that more stem mothers would chose to establish their galls at the top of the petiole, near the base of the leaf. The frequency distribution of gall positions on the petiole (Fig. 3.3.17) supports this notion: most stem mothers did prefer to locate their gall at the top of the petiole.

Did the gall location on the petiole influence aphid fitness? The position of the gall along the petiole had no effect on either gall weight (Fig. 3.3.18), or reproductive success (Fig. 3.3.19). Although there was no difference in petiole length between those with successful or failed galls ($F_{1,302} = 0.18$, n.s.), the closer the gall to the leaf, the less likely it was to abort (Fig. 3.3.20). The length of the petiole did not influence whether it was more likely to be occupied by 1, 2 or 3 galls ($F_{2,358} = 0.57$, n.s.).

To summarize the influences on fitness components, diagrams were constructed (Fig. 3.3.21a-f). In order to maintain legibility, only significant pathways were included. The number of leaves per shoot, leaf width, petiole length and number of galls per shoot all made a positive contribution to gall location on the shoot. However, the number of galls per leaf had a negative effect. Leaf width and number of galls per shoot both made a positive contribution to the number of galls per leaf, whereas the position on the shoot had a negative effect. The tree characteristics resulting in a direct contribution to gall weight were number of leaves per shoot and leaf length.

The diagrams and previous studies (e.g. Whitham, 1978) have indicated that there may be an important link between the leaf area available for photosynthesis and the fitness or reproductive success of aphids. This hypothesis was tested with the three treatments applied to single galled leaves, reducing the surface area of the leaf. I predicted that the greater the damage to the surface area of the leaf, the smaller the gall and the fewer the offspring. This prediction proved to be correct. Increasing damage to the photosynthetic leaf area produced a reduction in *P. spyrothecae* gall weight (Fig. 3.3.22) and in reproductive success (Fig. 3.3.23).

3. 4 Discussion

The mosaic of habitats that confronts an aphid influences the basic components of its life history, namely; reproduction, growth and development, and migration and dormancy (Solbreck, 1995; Hartnett & Bazzaz, 1984). Many aspects of leaf life history, such as longevity, age-specific assimilative activity, texture, chemical composition and demography affect patterns of herbivory, and in return insect activity may significantly alter the demography of leaves or other plant parts (Hartnett & Bazzaz, 1984).

The increase in photosynthate yields with leaf area is a fundamental property of all plants, and as this study shows, the total leaf area of the *Populus* x *canadensis* shoot on which the *Pemphigus spyrothecae* stem mother settles is important in terms of increased fitness. As predicted poplar shoots containing many leaves supported heavier galls with more aphids than those found on smaller shoots. The quantity of available resources for population growth is important in many insect systems. For instance, leaf folder sawfly (*Phyllocolpa* sp.) density was positively correlated with mean arroyo willow shoot length (Fritz & Nobel, 1989), and although the shoot size of wild grape (*Vitis arizonica*) did not affect the percentage survivorship or mean fecundity of the female leaf-galling grape phylloxera *Daktulosphaira vitifoliae*, populations of phylloxera were significantly higher on longer shoots than on short shoots among clones (Kimberling & Price, 1996). Poplar shoots without *P. spyrothecae* galls were smaller (i.e. had fewer leaves) than those with one or more galls. Similarly, arroyo willow shoots without sawflies were also significantly smaller than those shoots on the same clone with galls, indicating that leaf folders prefer longer shoots (Fritz & Nobel, 1989). The highest percentages of aborted or failed *P. spyrothecae* galls were found on small shoots. This coincides with Whitham's (1978) study in that as leaf size increased, the percentage of stem mothers that died during the colonisation attempt decreased.

If a higher reproductive fitness can be achieved by selecting the larger shoots or leaves, why are galls most often found on medium sized shoots? If fewer larger shoots or leaves are available to colonise, enhanced competition for these select sites may

force the stem mother to accept a lower reproductive output, which she would have increased through selecting larger shoots or leaves, in favour of a greater probability of survival on medium sized ones. I found that poplar shoots which develop leaves with a total surface leaf area of between 50-75 cm^2 occurred much more frequently than those attaining either a total leaf area size of between 75-100 cm^2 or those measuring between 25-50 cm^2

Aphids act as sinks, by diverting nutrients away from distant parts of the host plant and this sink-source relationship between the plant and its herbivore is strongly dependent on the site chosen by the foundress (Way & Cammell, 1970; Larson & Whitham, 1991). On *Pistacia* the apical part of the shoot was more likely to be galled by *Forda formicaria*, with leaves 9 and 10 the most crowded (Wool & Bar-El, 1995). *P. spyrothecae* fundatrices also preferred galling on the upper part of the shoot (Fig. 3.3.8). In contrast, *Adelges cooleyi* is rarely found in the top whorls of Engelmann spruce shoots which carry an almost zero survival rate. However, those fundatrices that do survive, grow to at least twice the size of the fundatrices colonising the bottom whorls, where survival is at least 60% (Fay & Whitham, 1990). The number of progeny and their growth rate is also at least double that of those in the bottom whorls. The vigorous growth of the top shoots must enhance female growth and reproductive fitness, but the earlier growth of this shoot results in high mortality rates due to seasonal low temperatures. As there were no real differences in reproductive outcome with position on the poplar shoot, this suggests that *P. spyrothecae* aphids are efficient at translocating nutrients from adjoining leaves.

Some authors (e.g. Rhomberg, 1984; Burstein & Wool, 1993) suggest that budburst phenology is a very important clue to unraveling population dynamics. The distribution of galls on a shoot can be explained by the availability of suitable leaves at the time of stem mother hatching. When the fundatrices emerge, the smaller basal leaves of shoots may already be too old for successful gall initiation. However, this assumes that all shoots on a tree develop at exactly the same rate and that stem mothers emerge all at once. From my own observations, leaves of *Populus* x *canadensis* buds unfurl and expand over a space of time, so that a range of growth is

always available to hatching stem mothers. This would explain why *P. spyrothecae* aphids gall on all leaf positions on the shoot.

Exploitive competition can affect galls on more distant parts of the plant, and indeed entire shoots may be impacted by the presence of galls on only a few leaves within the shoot (Larson, 1998). Figs. 3.3.5 and 6 illustrate how competitive interactions for photosynthetic assimilates causes a reduction in both gall weight and reproductive fitness when stem mothers share poplar shoots. The outcome of close competition, when galls share the same petiole, is addressed in a later chapter.

Many components of fitness correlate with leaf size and individuals that succeed in galling on the largest leaves benefit by having a greater probability of survival, a decreased risk of predation due to faster rate of gall development and more numerous and larger progeny containing more preformed embryos for the next generation. For instance, in some cases leaf length was correlated with sawfly density. Females may be able to assess the chemical and/or physiological properties of the leaves on the plant when they probe the underside of new leaves before ovipositing (Fritz & Nobel, 1989), and the average *P. betae* fundatrix selected cottonwood leaves 60% greater than the mean leaf size of the tree, resulting in an increase in fitness 7.3 times greater than on small leaves (Whitham, 1978). Mortality on the smallest leaves was 80% compared to 0% on the largest. Apart from the rate of gall failure, these results concur with my study on fitness in relation to leaf size. The strong selection pressure in favour of large leaves may have lead to the evolution of strategies and mechanisms by which the stem mother locates and colonises these leaves with considerable accuracy.

When the photosynthetic leaf area of galled petioles was reduced (leaf lamina cut, removed or left untouched), both *P. spyrothecae* gall weight (Fig. 3.3.22) and reproductive fitness (Fig. 3.3.23) decreased, illustrating the importance of photosynthates for colony growth. Stem resources are required for early *P. betae* gall growth, and as leaves become photosynthetically mature, ^{14}C is transported to the aphids. Once stem mothers are encased in their gall, they are immobile, thus manipulation of normal transport patterns appears crucial in maintaining food resources. Although an individual leaf can successfully support numerous galls and

still be photosynthetically active, gall tissue has limited photosynthetic ability, and may cause an increase of photosynthesis in other parts of the leaf (Steffan & Rilling, 1981). The gall eventually causes the leaf blade to become chlorotic, thus reducing photosynthesis (Williams & Whitham, 1986). It is interesting to note that Hartnett & Bazzaz (1984) determined that peak densities of aphids occur near leaves that are photosynthesising at only 77% of their maximum rate.

Could the selective settling of stem mothers cause the leaf to become larger through the aphids' feeding activities? Whitham (1978) examined the size of leaves with similar potential for becoming large and found no significant differences between galled and ungalled leaves. Feeding activities of these aphids do not appear to affect mature leaf size, but this is not always the case in other insect systems. For instance, *Phytoptus emarginatae*, an eriophyid mite galling the leaves of wild plum, reduces the size of neighbouring leaves within a shoot relative to leaves that develop without nearby galls (Willson & O'Dowd, 1990).

For vascular feeders the base of the leaf blade (junction with petiole) is an important site as all substances translocated into or out of the leaf must flow by this position. *Pemphigus* aphids respond to the increasing demand for resources to feed a growing population in the gall by importing resources from neighbouring leaves (Whitham, 1978; Larson & Whitham, 1991; Burstein, Wool & Eshel, 1994). By positioning a gall near the junction between the leaf and petiole, where the probability of survival is higher, *P. spyrothecae* galls are able to act as effective resource sinks. Although *P. spyrothecae* gall position on the petiole had no effect on the number of offspring produced, basal *P. betae* galls generate 65% more aphids than distal galls on cottonwood leaves (Whitham, 1986). Perhaps basal galls exert a stronger mobilising effect on ^{14}C fixed in the neighbouring leaves (hence the importance of a large shoot area) whilst distal galls have a very restricted control over translocation. The pattern of gall development by the wasp *Cynips divisa* on multi-gall veins corresponded to the resource-interception theory which suggests that plant resources required by distal galls for growth may be intercepted to a certain extent by proximal galls, causing an increase in the size of the latter (Sitch, Grewcock and Gilbert, 1988). In contrast, there was no within-leaf differences in phylloxera survivorship among vein type or region

of leaf colonised, and although females on secondary veins had more offspring than those on primary or tertiary veins, this accounted for only 3% of the variance in fecundity, and suggests that individual phylloxera perform equally well at all within leaf sites (Kimberling & Price, 1996).

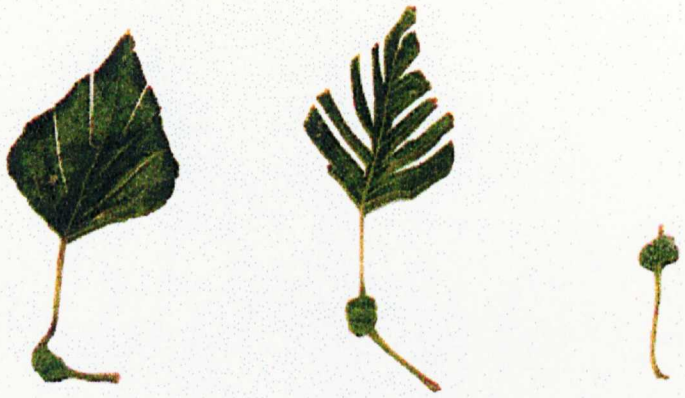
When selecting a leaf at budburst, *Pemphigus* aphids may be responding to the growth rate of a leaf, rather than its size or nutritive quality, and since growth is faster at the base of the leaf (Mohr & Schopfer, 1995) this could account for the aphids' choice of basal galling sites. Hormones such as auxin (indole -3- acetic acid, IAA) and cytokinin are important in leaf growth (Bidwell, 1974). The concentration of auxin available to tissues can have a determining effect on cell growth and differentiation. IAA influences linear growth in leaves and therefore leaf area and certain aspects of leaf shape. Cells may elongate at a constant rate for a considerable time and proceeds by a series of viscoelastic extensions (an initial rapid extension followed by a further slower extension) driven by the turgor pressure of the cell sap (Wareing & Philips, 1978). As aphids feed either by using the turgor pressure in the sap in the sieve tubes in the phloem tissue or by using their cibarial pump (Dixon, 1985), this may be a way for aphids to determine the quality of young developing leaves. Although females of the galling midge *Giraudiella inclusa* on *Phragmites australis* did not appear to distinguish between shoots when ovipositing, the chosen site of ovipositing females and migrating first instar larvae was predictably high in nutritional content, such as nitrogen, sugar and minerals (Tscharntke, 1988). Phloem sap is rich in sugars and as large leaves also have a rapid growth rate, the quantity of nutrients flowing into these leaves, and thus turgor pressure, may be higher.

Aphids reportedly have an acute phytochemical sensory ability suggesting that the local distribution of plant chemicals plays an important part in the habitat selection process. Substances like phenols and tannins vary in their distribution on every scale of the plant: from branch to branch, shoot to shoot, leaf to leaf and within leaves. The direct measurement of secondary plant chemicals would be the ideal investigative method, but how much of these substances the aphid itself is exposed to is difficult to determine. Due to the strong compartmentalisation of secondary plant chemicals in plant tissue (Conn, 1984) aphids may avoid ingesting most of them (especially

because aphids tend not to puncture cells as they insert their stylets to reach the sieve tubes). *Pontania* gall tissues generally contain lower levels of nitrogen and higher levels of phenolic compounds than surrounding ungalled tissue (Hartley, 1998). In two *Salix* species, phenolic biosynthesis in the gall tissue reduced as the season progressed, whilst phenolic levels increased in the adjacent plant material. Interestingly, if the gall inhabitants are killed with insecticide, phenolic levels in the gall tissue revert to the same levels as those in surrounding plant tissue. The total concentration of phenols in shoots may also help explain the distribution pattern of *Pemphigus* aphids on cottonwoods (Zucker, 1982). Within-shoot variation in total phenols may be an important factor affecting leaf choice. *P. betae* stem mothers galled on leaves that had lower concentrations than on ungalled leaves. Zucker also determined that leaves have a phenolic gradient such that the basal part of the leaf, where *P. betae* stem mothers exhibit the highest survival rate and achieve the greatest fitness (Whitham, 1978) contains the lowest concentrations. How secondary compounds influence *P. spyrothecae* galling site selection remains unknown, but this would be an interesting addendum to this study.

Many other factors can also influence the choice of galling site. Adverse weather conditions such as rain may reduce the number of aphids at budburst (Dixon, 1976) and although few insect predators are present at this time of year, bird predation may also play an important role in adjusting aphid population numbers. It is impossible to unravel the whole story, and the use of path diagrams can only suggest possible causal factors. As Figs. 3.3.21a-f illustrate, *Pemphigus spyrothecae* live in a complex micro-environment, where certain factors play a more important role than others in determining aphid fitness.

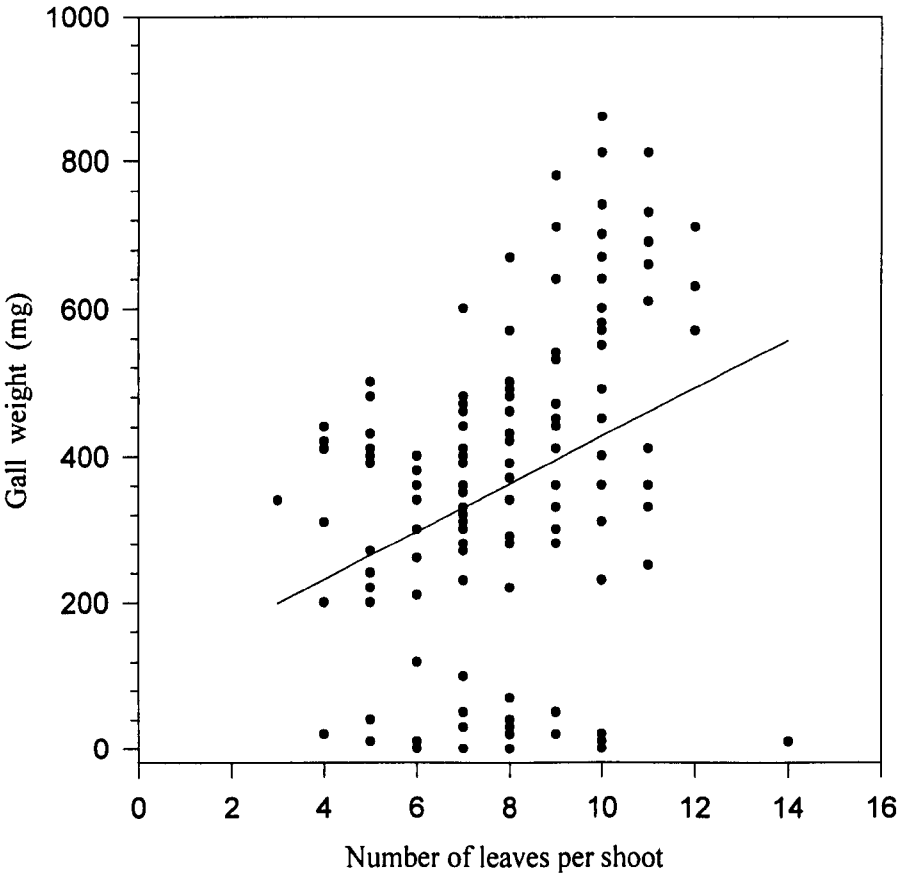
Plate 3. 2. 1



Galled *Populus* x *canadensis* leaves were subjected to one of three treatments; cuts in the lamina, removal of lamina and the total removal of the leaf, in order to ascertain how the quantity of photosynthetic leaf area available effects gall growth and reproductive fitness of the galling aphid *Pemphigus spyrothecae*.

Figure 3. 3. 1

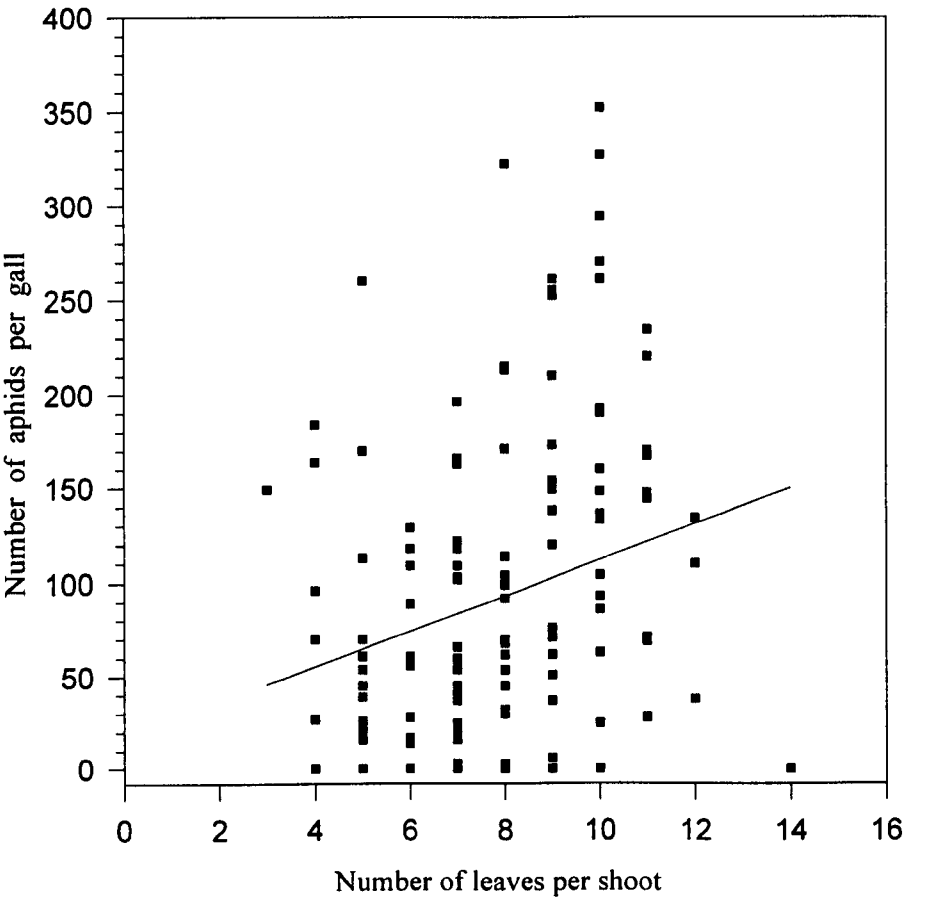
The effect of number of leaves per shoot on *Pemphigus* gall weight



On singly-galled shoots, a greater number of leaves per poplar shoot produced heavier *P. spyrothecae* galls ($r^2 = 0.104$, $F_{1,132} = 15.27$, $p < 0.001$).

Figure 3. 3. 2

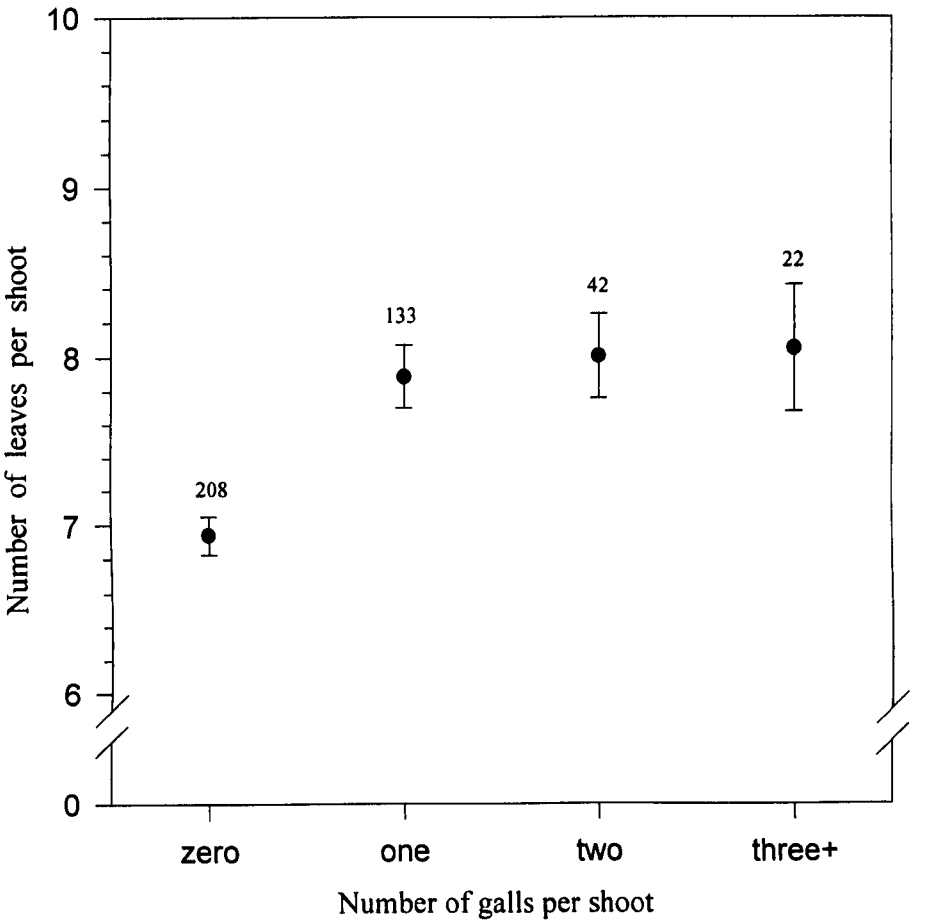
The effect of number of leaves per shoot and aphid reproductive outcome



On singly-galled shoots, a greater number of leaves per poplar shoot produced more *Pemphigus spyrothecae* aphids per gall ($r^2 = 0.06$, $F_{1,132} = 8.41$, $p < 0.01$).

Figure 3. 3. 3

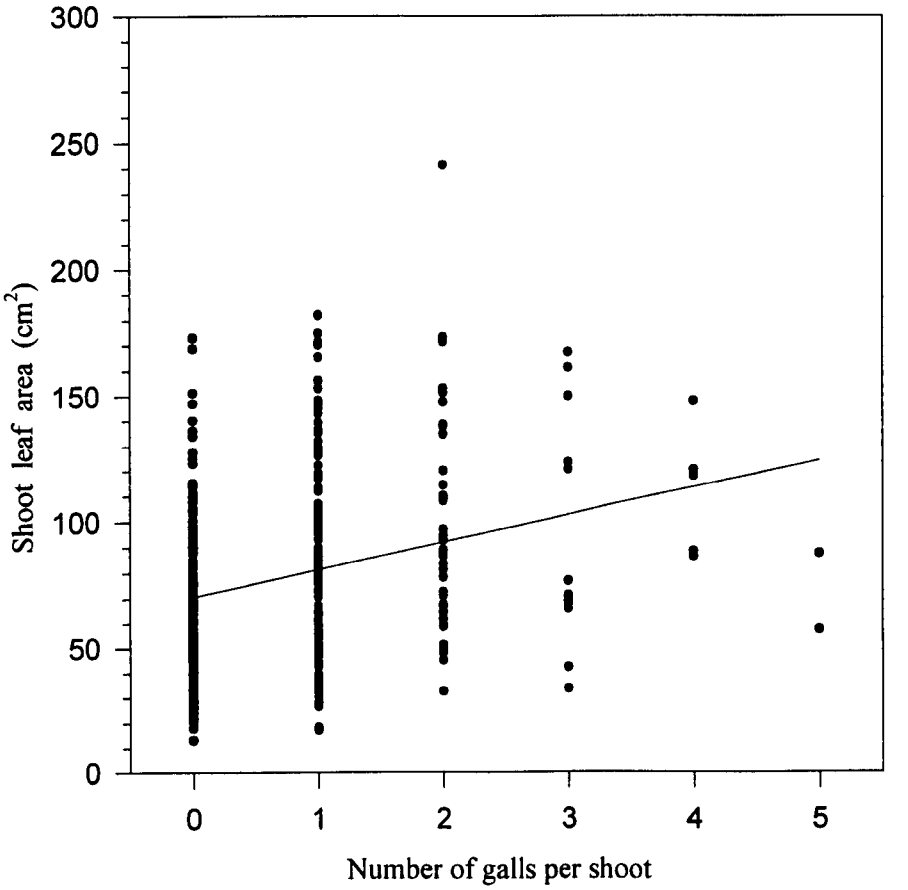
Poplar shoot size with *Pemphigus* galls present or absent



Poplar shoots without *P. spyrothecae* galls tended to have fewer leaves per shoot than those with galls (Kruskal - Wallis $H = 29.10$, $p < 0.001$).

Figure 3. 3. 4

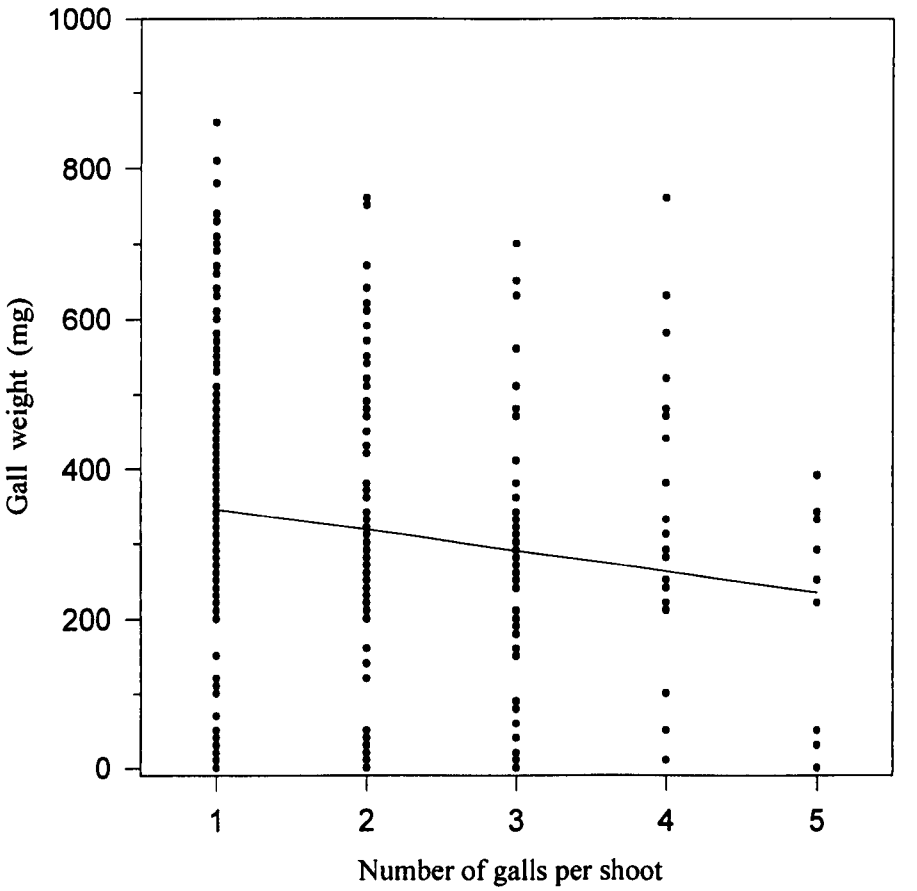
Poplar shoot leaf area with *Pemphigus* galls present or absent



There were more galls located on poplar shoots of a greater shoot surface leaf area ($r^2 = 0.08$, $F_{1,404} = 34.15$, $p << 0.001$).

Figure 3. 3. 5

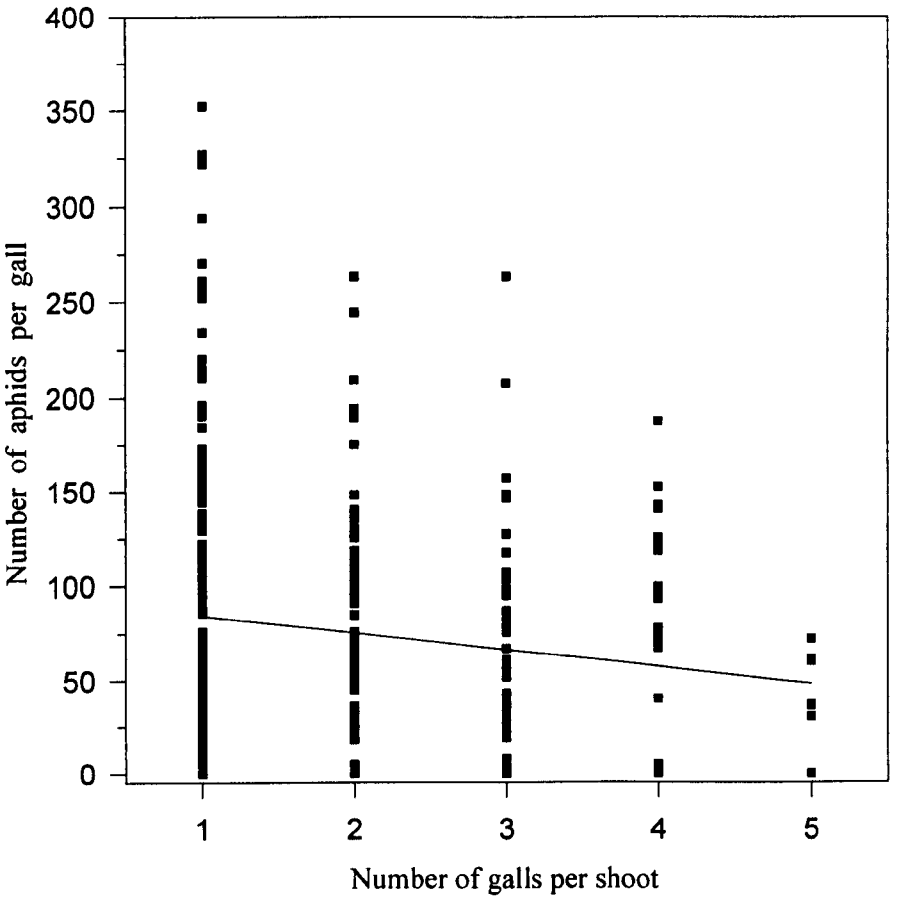
The effect of sharing poplar shoots with other *Pemphigus* galls on gall weight



Relationship between the number of *P. spyrothecae* galls per shoot and gall weight. Gall weight decreased as the the number of galls per poplar shoot increased ($r^2 = 0.02$, $F_{1,317} = 6.49$, $p < 0.05$).

Figure 3. 3. 6

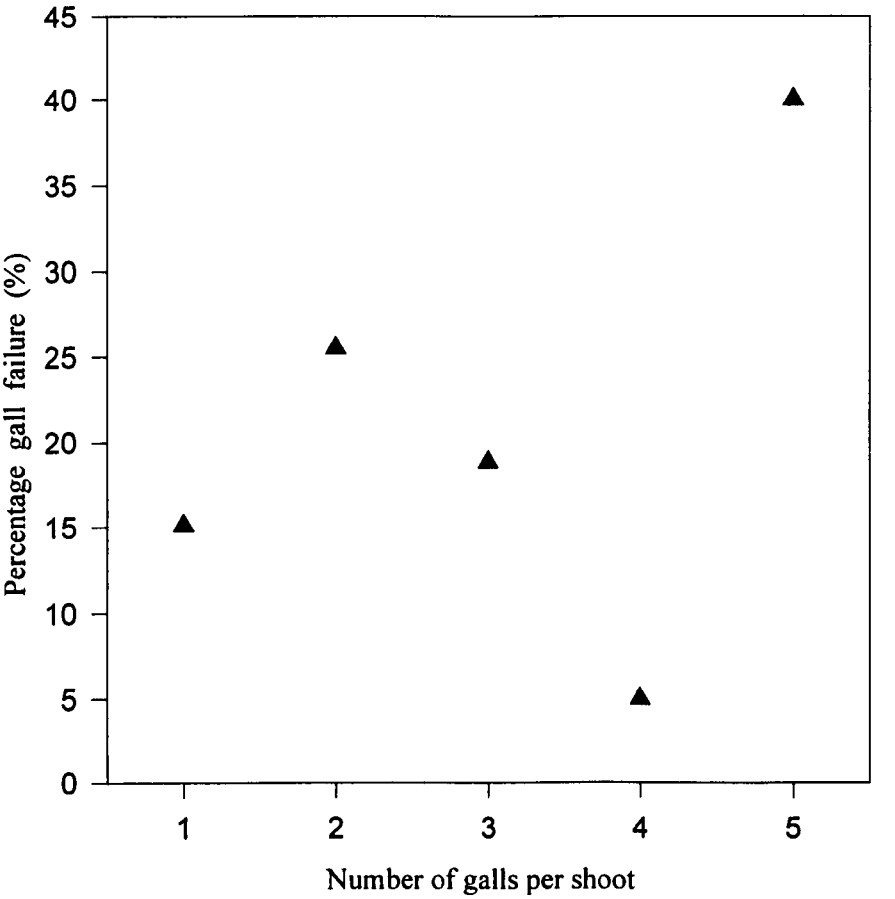
The effect of sharing poplar shoots with other *Pemphigus* galls on reproductive success



Relationship between the number of galls per poplar shoot and reproductive outcome of *P. spyrothecae*. As the number of galls increased, the number of progeny decreased ($r^2 = 0.02$, $F_{1,317} = 6.02$, $p < 0.05$).

Figure 3. 3. 7

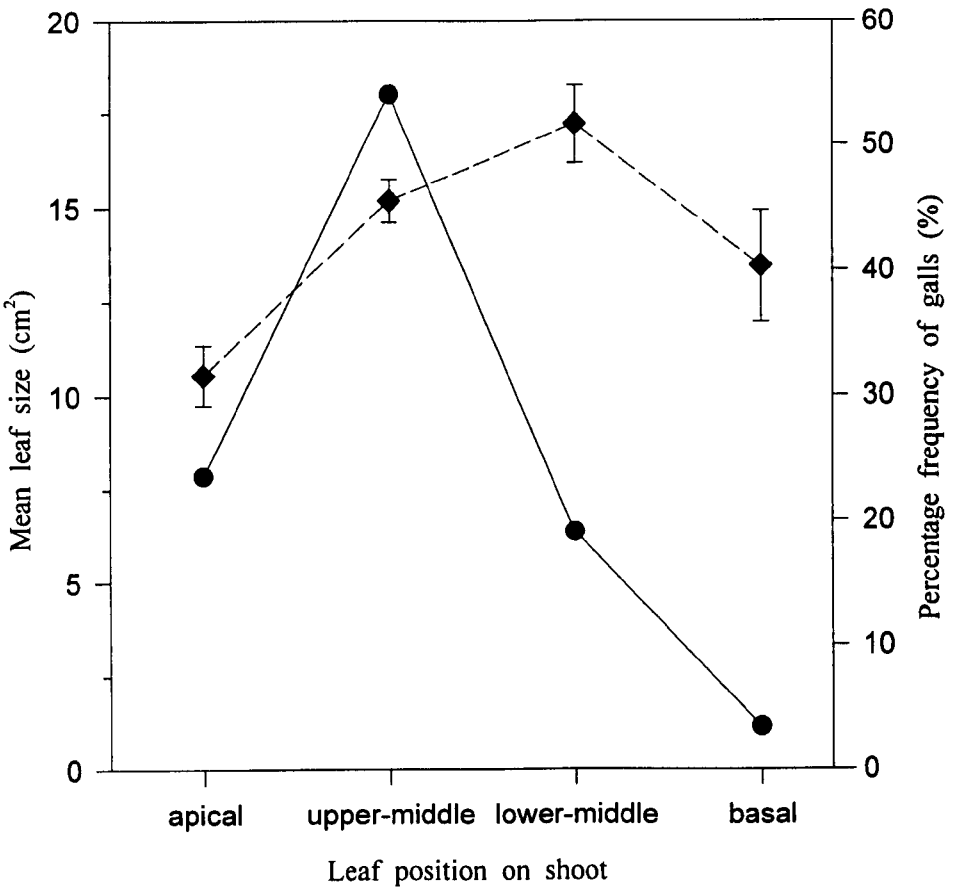
The effect of shoot sharing on gall failure



Relationship between the number of *P. spyrothecae* galls per shoot and gall failure. There was a significant difference between the number of galls per poplar shoot (χ^2_4 = 9.53, $p < 0.05$) and gall failure.

Figure 3. 3. 8

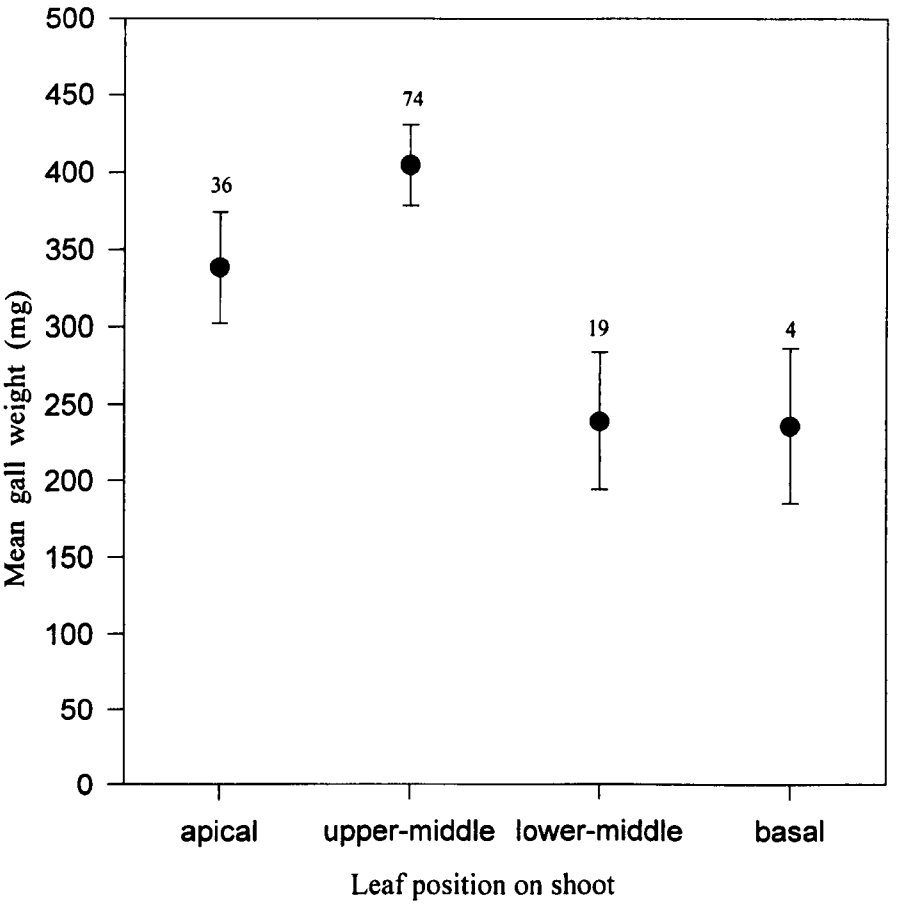
The effect of leaf size on the position of galls on the shoot



The poplar leaves on the upper-middle section of the shoot were the most frequently galled. However, these do not necessarily contain the leaves which will have the largest surface leaf area when mature. Size differed significantly with position on the shoot (ANOVA: $F_{3,235} = 10.03$, $p << 0.001$).

Figure 3. 3. 9

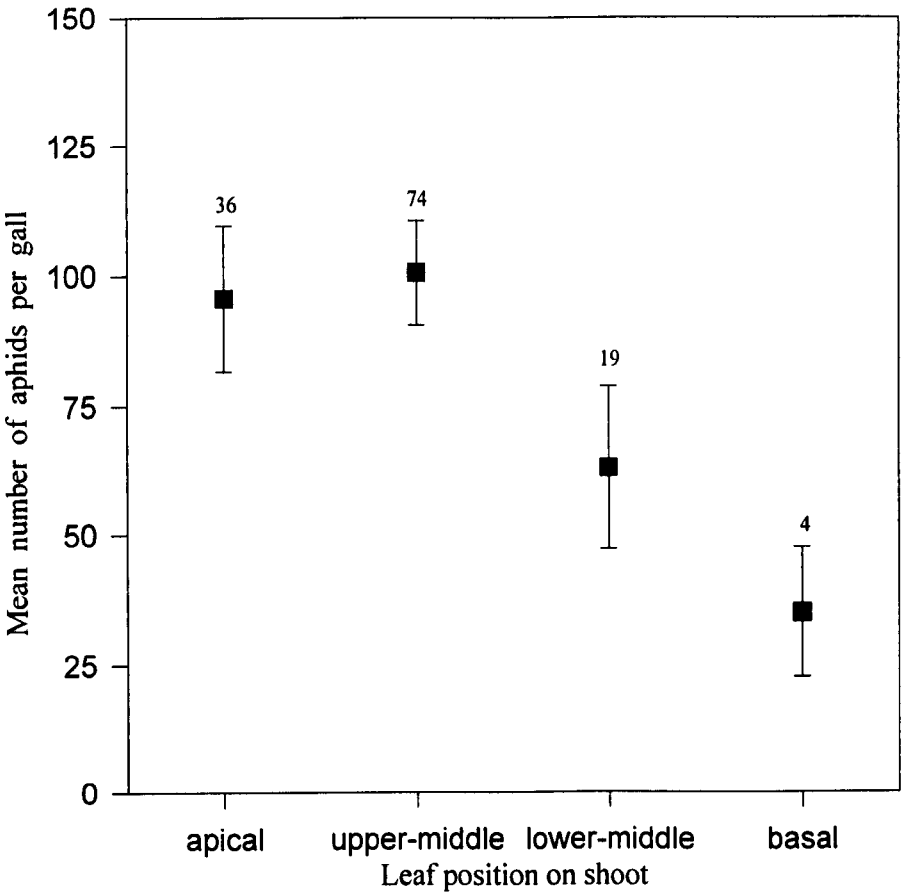
The effect of gall position on the poplar shoot on *Pemphigus* gall weight



Gall weights differed significantly with position on the singly-galled shoots (ANOVA: $F_{3,132} = 3.60$, $p < 0.05$).

Figure 3. 3. 10

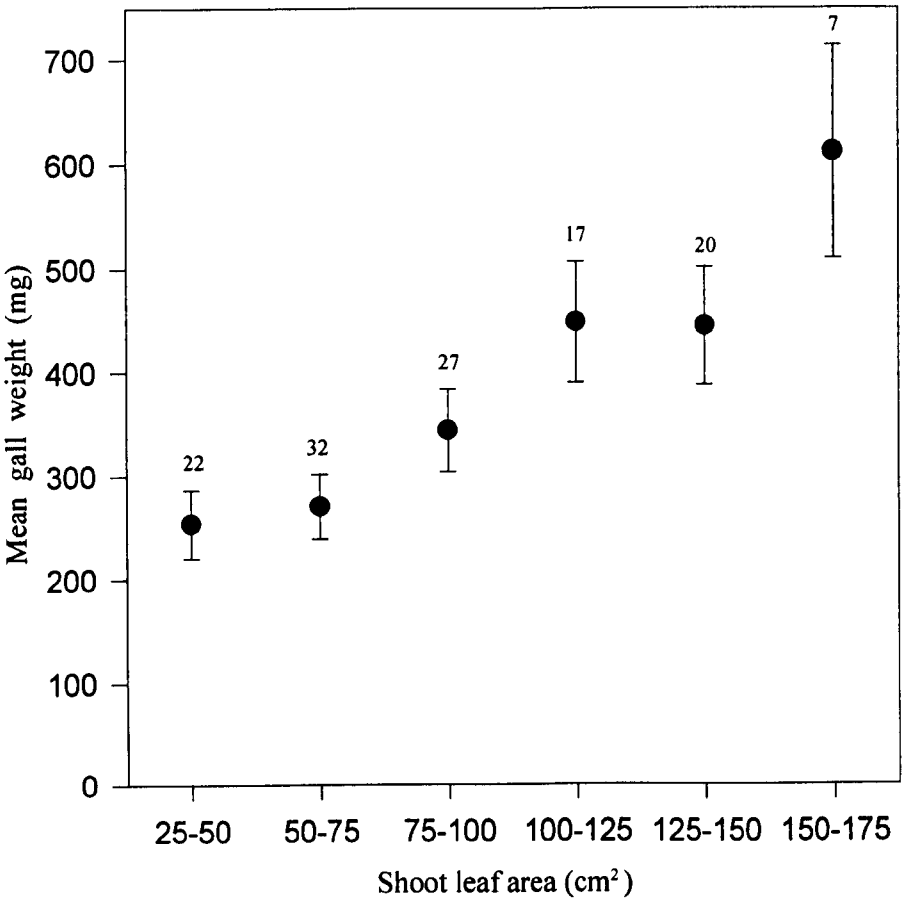
The effect of gall position on poplar shoots on *Pemphigus* reproductive success



There were no significant effects of position on the poplar shoot on aphid reproductive success (ANOVA: $F_{3,132} = 1.70$, n.s.).

Figure 3. 3. 11

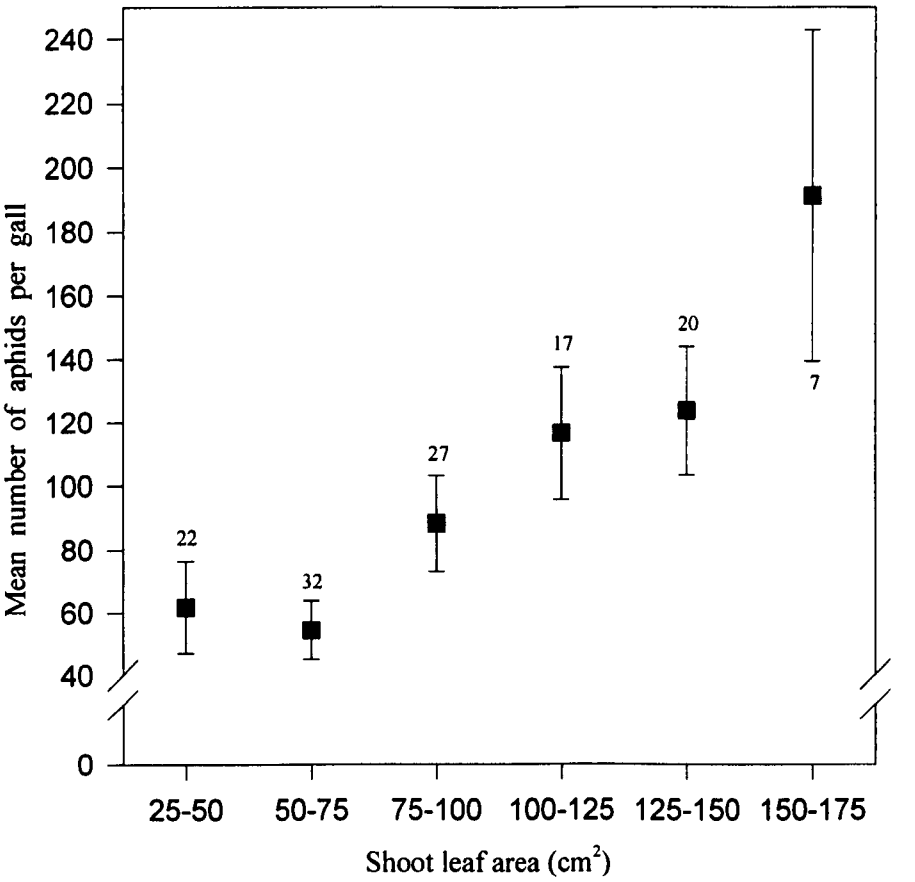
The effect of shoot leaf area on *Pemphigus* gall weight



Graph of mean aphid gall weight on different sized poplar shoots when only a single gall is present on the shoot. There were significant effects of shoot area category on gall weight (ANOVA: $F_{5,124} = 5.60$, $p << 0.001$).

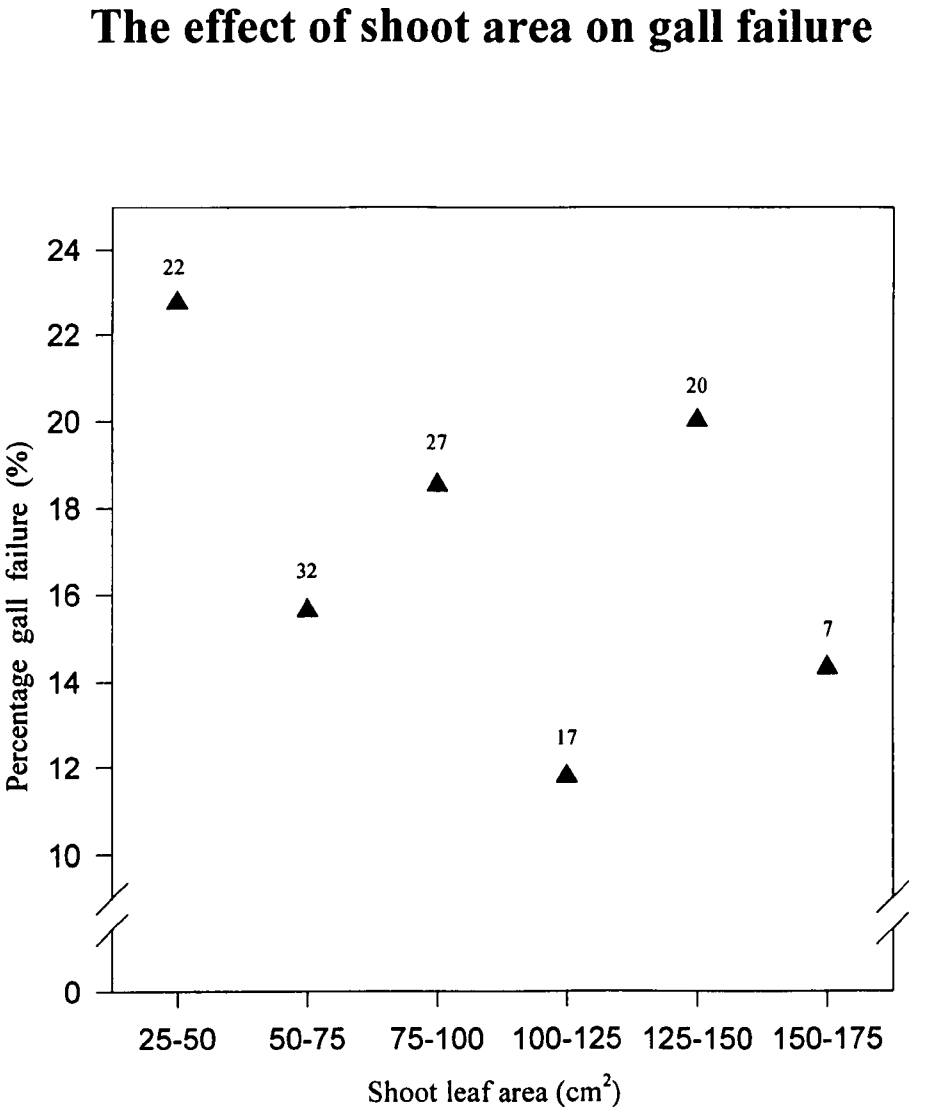
Figure 3. 3. 12

The effect of shoot leaf area on aphid reproductive success



Reproductive success of *Pemphigus* aphids on poplar shoots of different sizes but with only one gall. There were significant effects of shoot area categories on the number of offspring per gall (ANOVA: $F_{5,124} = 5.33$, $p << 0.001$).

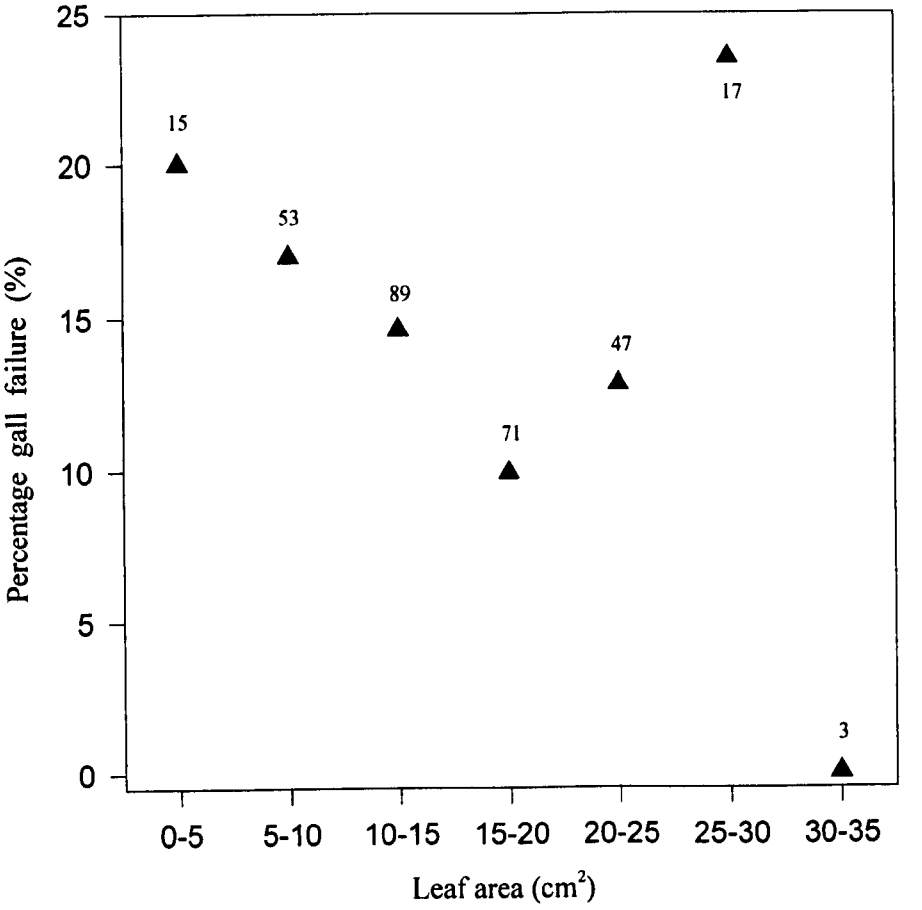
Figure 3. 3. 13



Graph of percentage *P. spyrothecae* gall failure on poplar shoots of different sizes, when only one gall is present on the shoot. There was no apparent difference between shoot-area category and gall failure ($\chi^2_5 = 1.03$, n.s.).

Figure 3. 3. 14

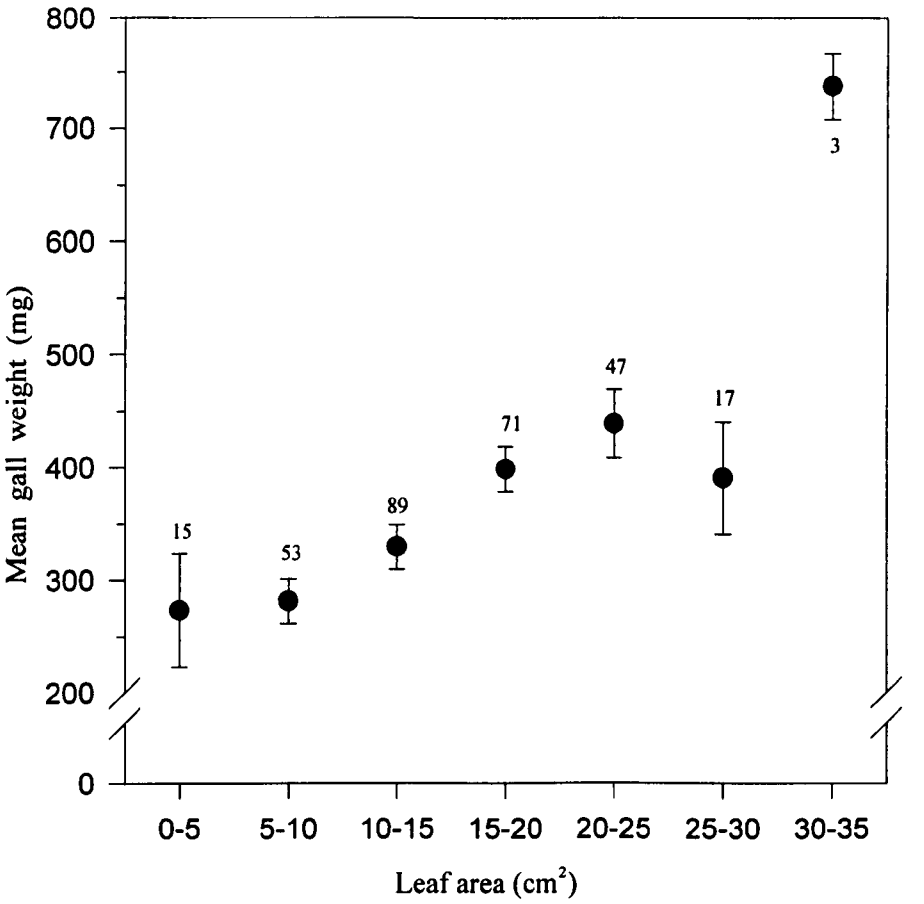
The effect of leaf area on *Pemphigus* gall failure



Graph of area of individual poplar leaves and gall failure for single galls. There was no significant difference between leaf-area category and rate of gall failure ($\chi^2_6 = 3.64$, n.s.).

Figure 3. 3. 15

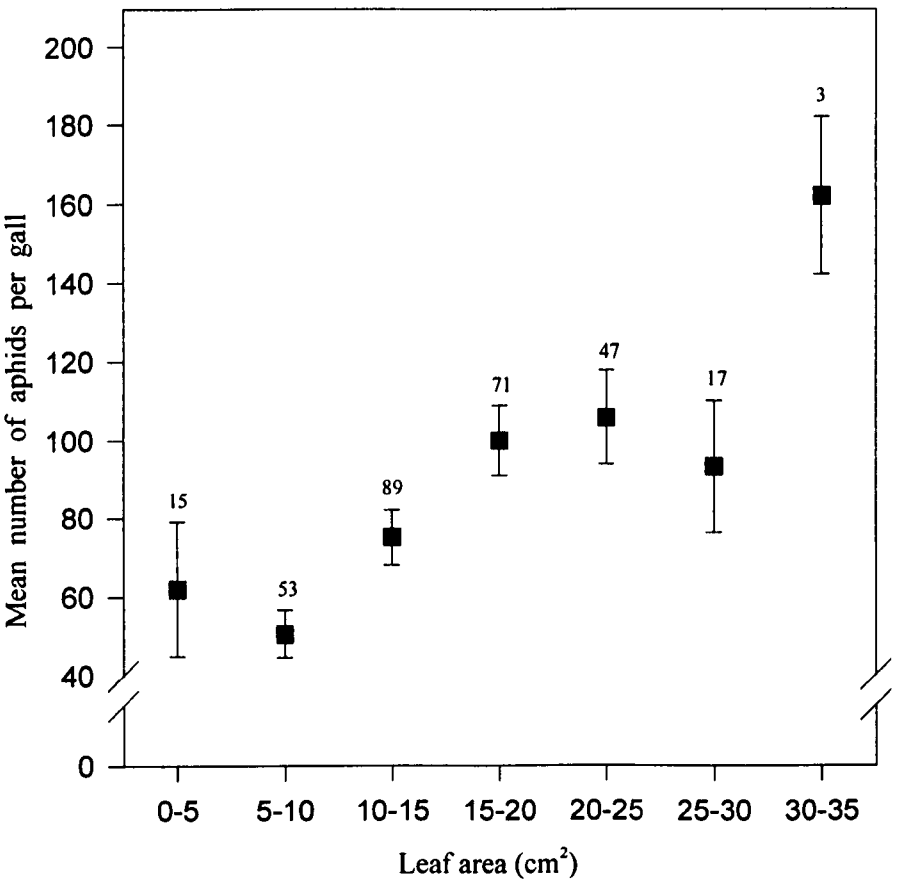
The effect of leaf area on *Pemphigus* gall weights



Graph of area of individual poplar leaves and gall weights on singly-galled petioles. There were significant differences among leaf-area categories and gall weights (ANOVA: $F_{6, 294} = 5.80$, $p << 0.001$).

Figure 3. 3. 16

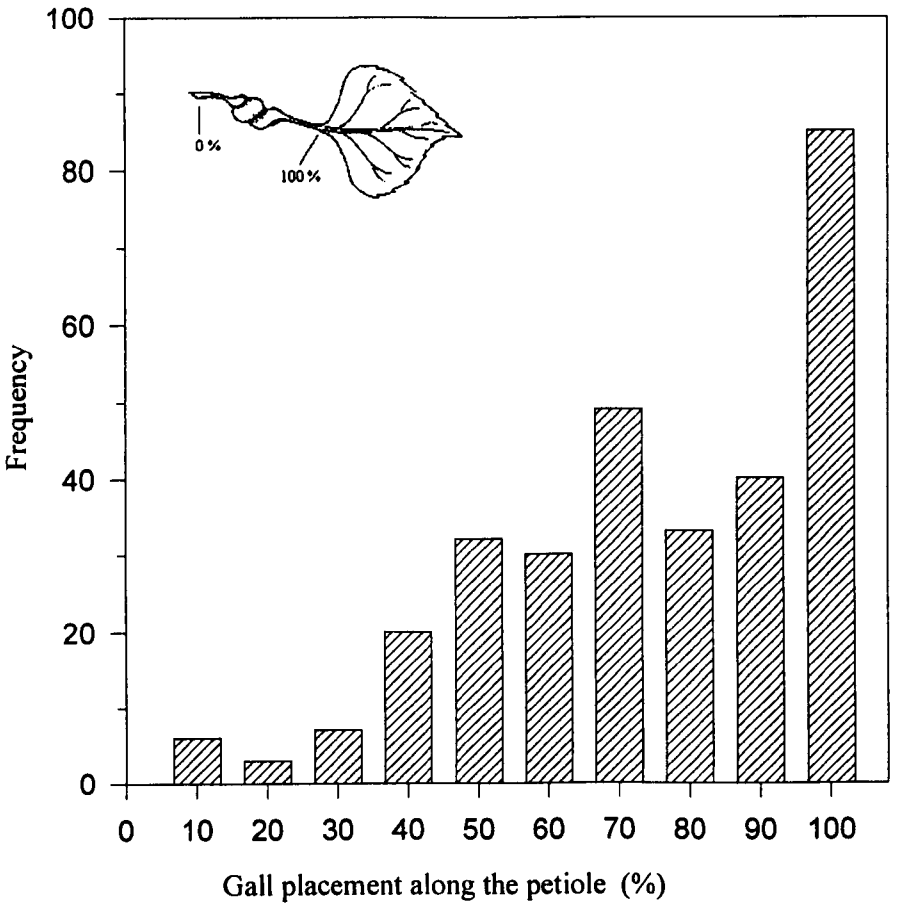
The effect of leaf area on aphid reproductive success



Graph of area of individual poplar leaves and *P. spyrothecae* reproductive success on singly-galled petioles. There were significant differences in leaf-area categories on number of progeny per gall (ANOVA: $F_{6,294} = 4.92$, $p << 0.001$).

Figure 3. 3. 17

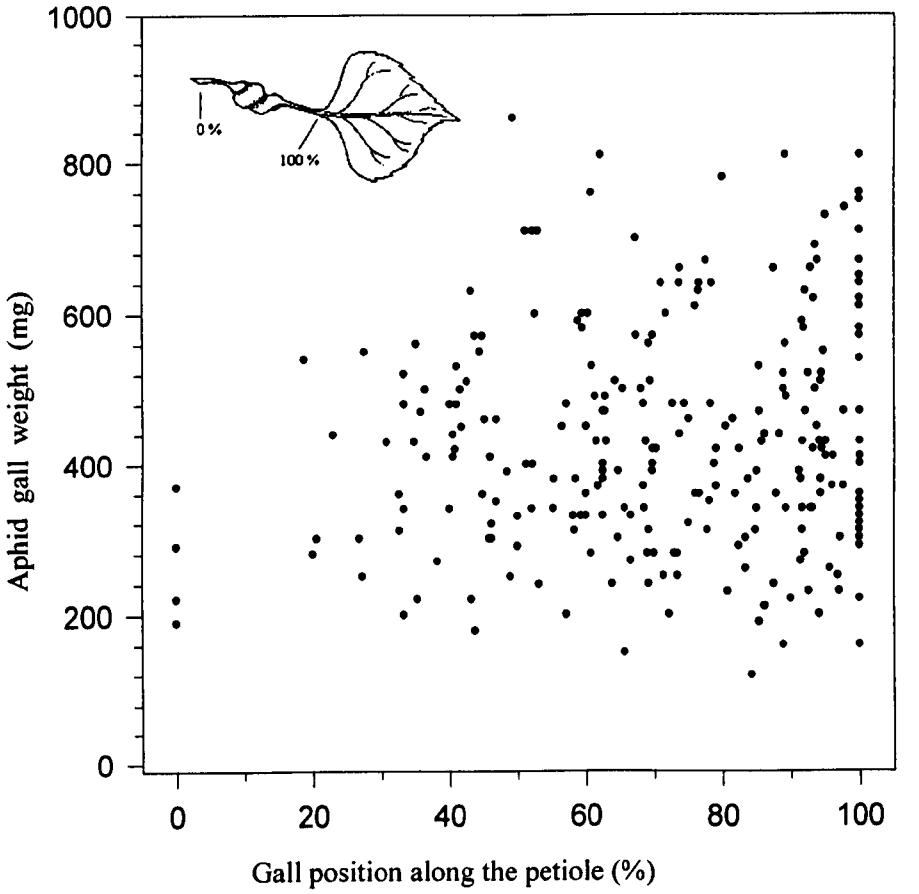
Frequency of galls occuring along the petiole as a percentage of the total length of the petiole



Frequency distribution showing the location of *P. spyrothecae* galls on poplar petioles, when only a single gall is present. Most galls are situated close to the base of the leaf (position is measured from the point of attachment of the petiole to the shoot).

Figure 3. 3. 18

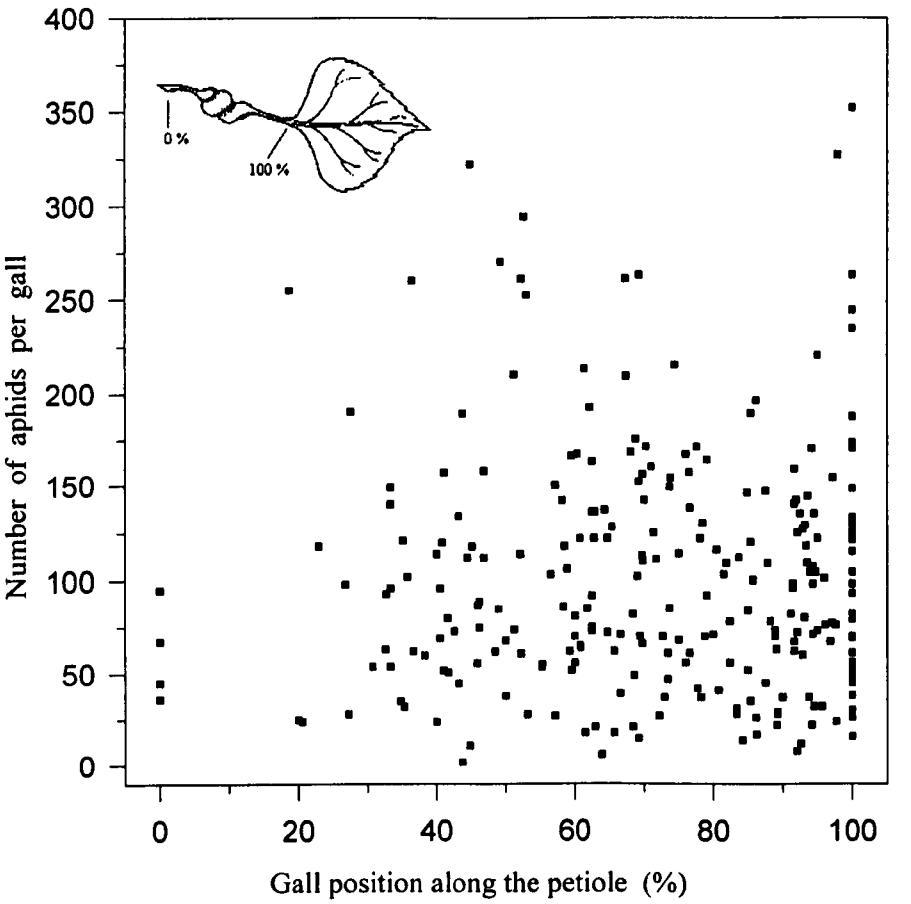
Gall weight at different positions along the petiole



There was no relationship between gall position (as a percentage of total length) and *P. spyrothecae* gall weight ($r^2 = 0.01$, $F_{1,254} = 2.59$, n.s.). Gall failures were excluded.

Figure 3. 3. 19

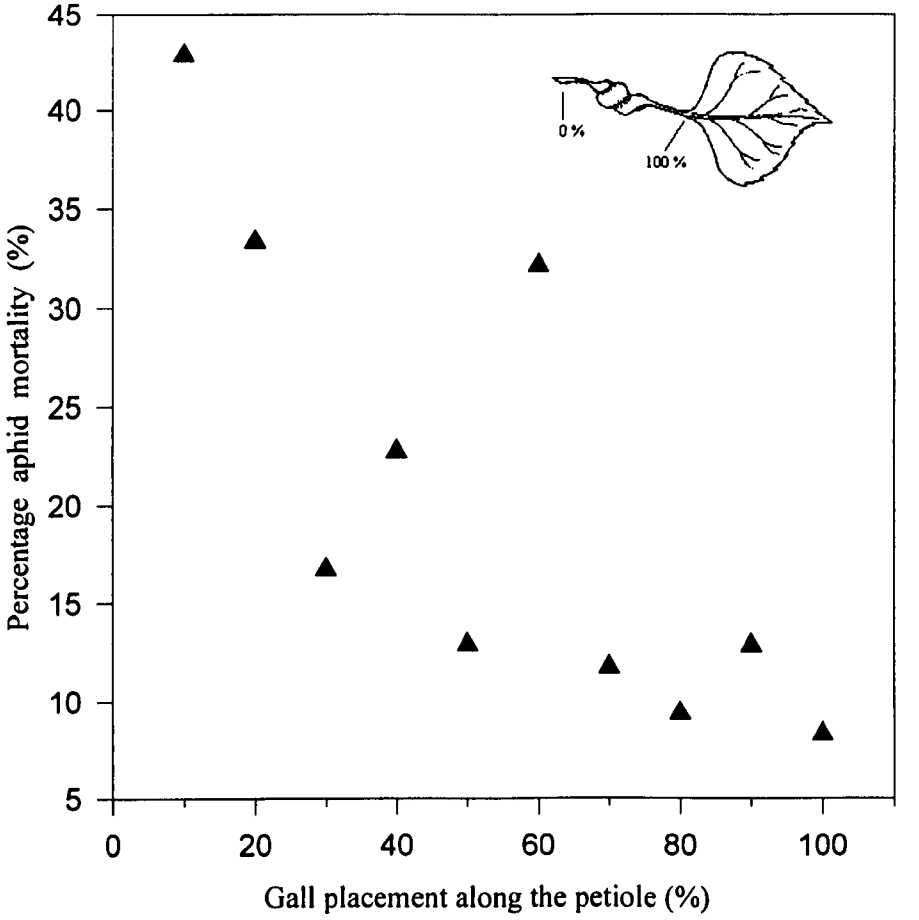
Aphid reproductive success at different positions along the petiole



There was no relationship between *Pemphigus* gall position (measured as a percentage of the total poplar petiole length from the point of attachment of the petiole) and reproductive success ($r^2 = 0.0001$, $F_{1,254} = 0.02$, n.s.). Gall failures were excluded.

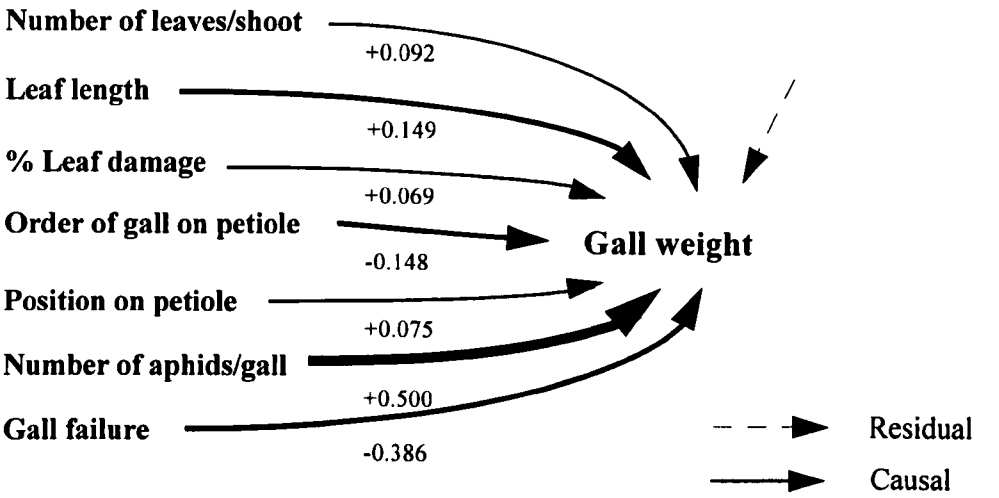
Figure 3. 3. 20

Gall failure at different positions along the petiole



Relationship between gall position on the poplar petiole (measured as a percentage of the total petiole length, from the point of attachment of the petiole) and gall failure. There was a significant association between *P. spyrothecae* gall position and gall failure ($\chi^2_9 = 17.35$, $p < 0.05$).

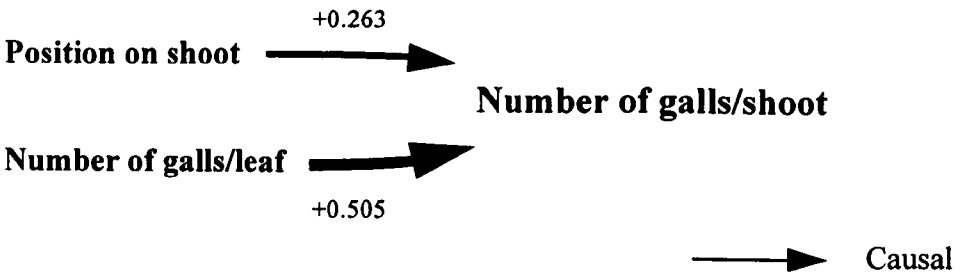
Figure 3. 3. 21a



Gall weight = 301.75 + 1.914 leaf length + 0.4844 leaf width - 91.641 order + 9.951 #leaves/shoot - 4.829 position on shoot - 0.6972 petiole length + 0.6071 position on petiole - 1.035 #galls/shoot + 8.413 #galls/leaf + 1.053 %leaf damage + 1.452 #aphids/gall + 202.16 gall failure

$R^2 = 0.7809$, $F = 68.26$, $df = 249$, $p < 0.0001$

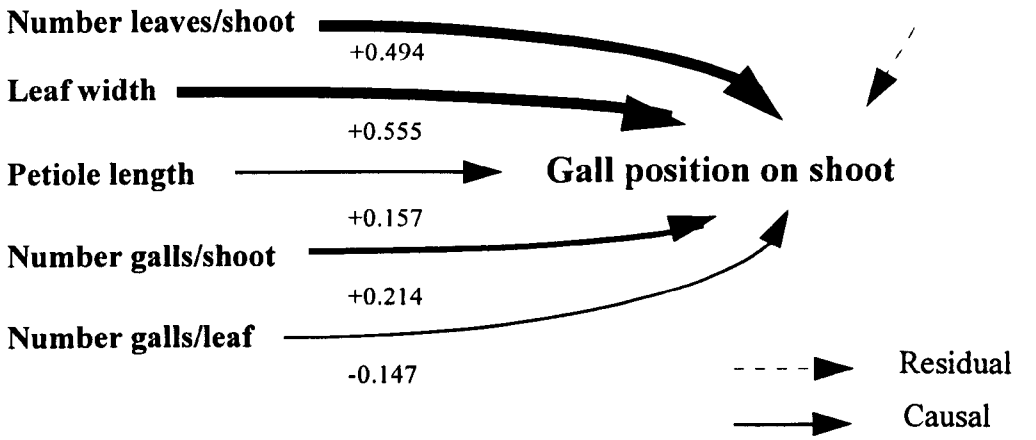
Figure 3. 3. 21b



Number of galls per shoot = 0.3859 + 0.2212 position on shoot + 0.0007 shoot area + 1.164 #lgalls/leaf

$R^2 = 0.3467$, $F = 36.751$, $df = 277$, $p < 0.0001$

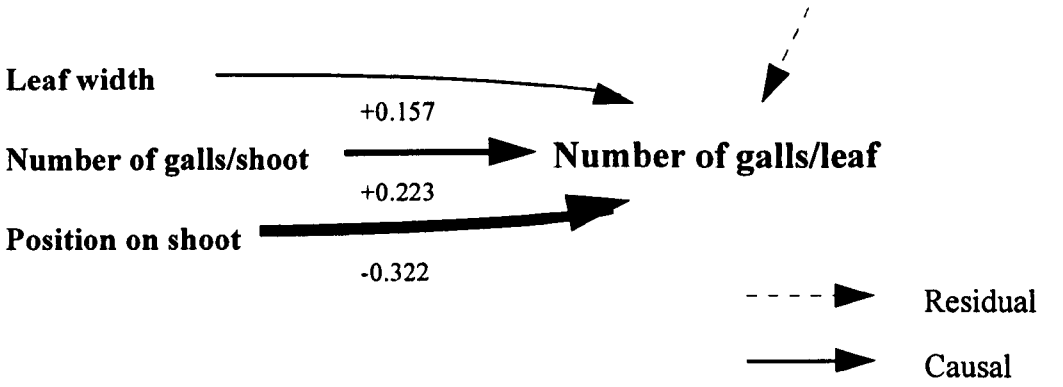
Figure 3. 3. 21c



Gall position on shoot = 0.5409 - 0.0089 leaf length + 0.0534 leaf width + 0.3263 #leaves/shoot - 0.0283 petiole length + 0.2579 #galls/shoot - 0.3994 #galls/leaf

$R^2 = 0.5814$, $F = 60.197$, $df = 260$, $p < 0.0001$

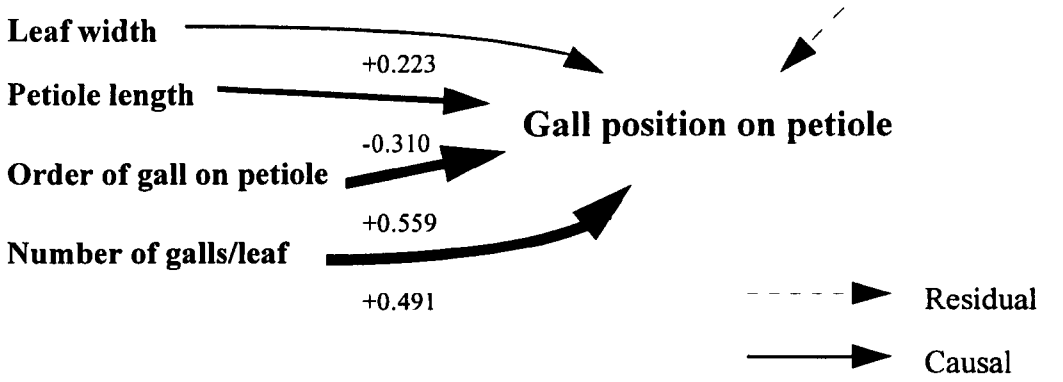
Figure 3. 3. 21d



Number of galls per leaf = 0.6571 + 0.0028 leaf length + 0.0046 leaf width + 0.0175 #leaves/shoot - 0.1189 position on shoot - 0.0012 petiole length + 0.2424 #galls/shoot

$R^2 = 0.3259$, $F = 20.95$, $df = 260$, $p < 0.0001$

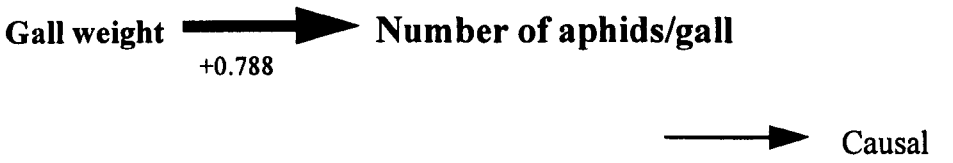
Figure 3. 3. 21e



Gall position on petiole = 59.453 - 0.7237 petiole length + 0.0861 leaf length + 0.4580 leaf width + 38.172 gall order on petiole - 24.885 # galls/leaf

$R^2 = 0.2809$, $F = 32.11$, $df = 411$, $p < 0.0001$

Figure 3. 3. 21f

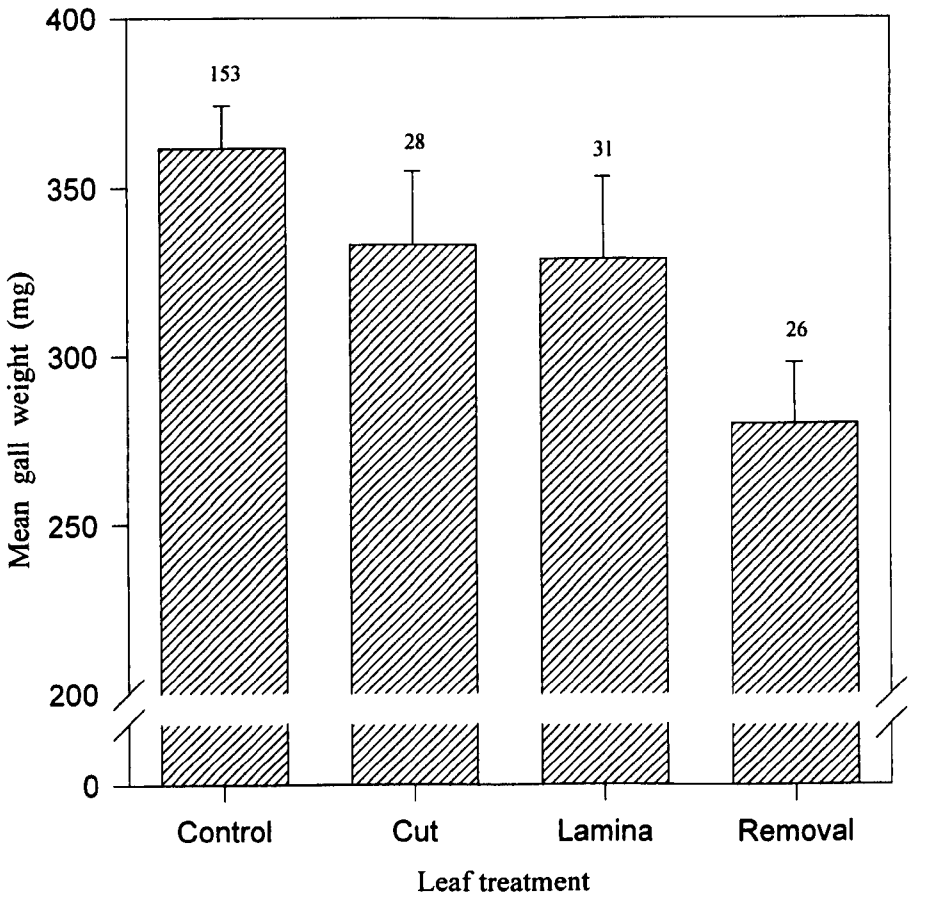


Number of aphids per gall = 17.955 + 0.2714 gall weight - 0.3309 leaf length + 0.111 leaf width + 17.783 order + 1.897 #leaves/shoot - 3.428 position on shoot + 0.6387 petiole length - 0.050 position on petiole + 0.7071 #galls/shoot - 12.273 #galls/leaf - 0.2372 %leaf damage - 8.135 gall failure

$R^2 = 0.6545$, $F = 36.286$, $df = 249$, $p < 0.0001$

Figure 3. 3. 22

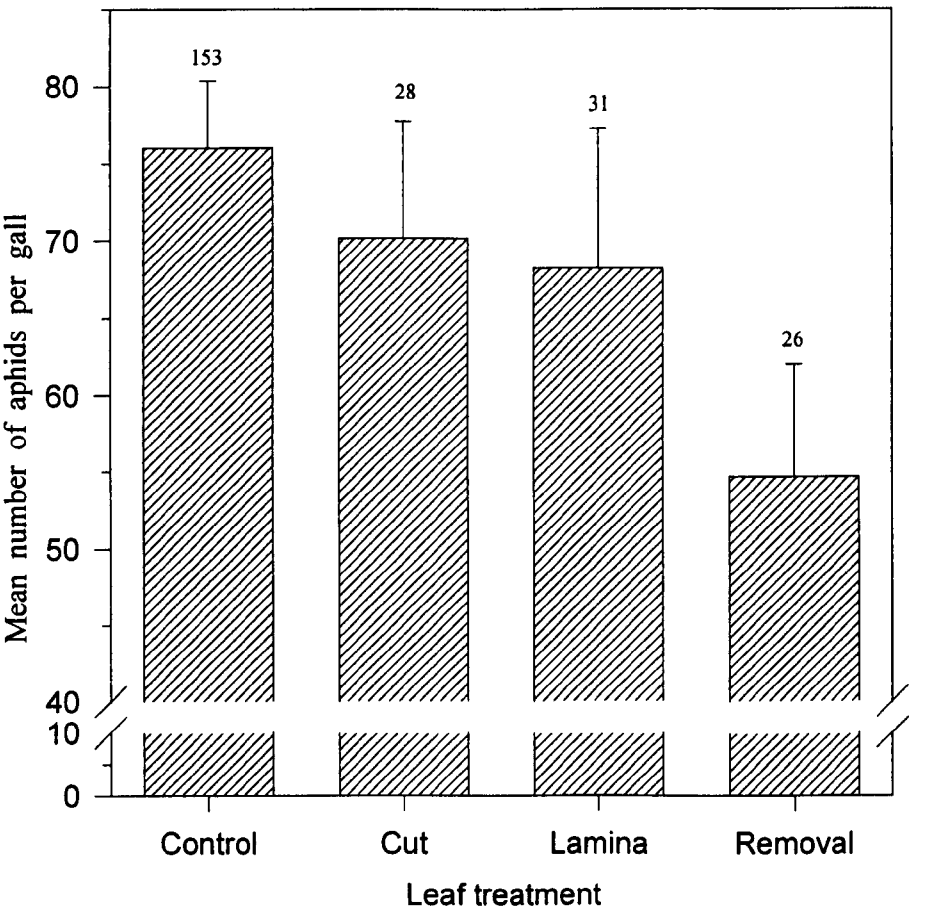
Gall weights on poplar leaves subjected to different treatments



As poplar leaf damage increased, the average *P. spyrothecae* gall weight decreased ($Z = 2.78$, $p < 0.05$).

Figure 3. 3. 23

Aphid reproductive success on poplar leaves subjected to different treatments



The number of progeny per gall decreased as leaf damage increased ($Z = 1.82$, $p < 0.05$).

Chapter 4

APHID EMERGENCE AT BUDBURST

4. 1 Introduction

The timing of host plant budburst varies within and among host individuals and within years (Dixon, 1976; Crawley & Akhterauzzaman, 1988; Mopper & Simberloff, 1995). In some studies, differences in insect performance and population density are related to budburst dates among individual trees (e.g. Kerslake & Hartley, 1997; Akimoto, 1998), whilst in others (e.g. Watt & McFarlane, 1991; Crawley & Akhteruzzaman, 1988) no consistent pattern of synchrony between budburst and insect hatch could be detected, thus calling into question the generality of tree phenology as a generator of the spatially patchy distribution of insects on their hosts.

In aphids where synchrony is evident, adaptation to the time of budburst of individual host trees may result in genetic differentiation in hatching time among tree-associated aphid populations (Komatsu & Akimoto, 1995; Hedrick, 1996). Genetic differentiation may have occurred because the insect has poor migratory abilities. In contrast, host-alternating aphids are highly mobile, and are more likely to experience unpredictable heterogeneity on the primary host, and so their development may be asynchronous to budburst (Moran, 1992).

Once a migrant has found an appropriate host, *Pemphigus betae* aphids will walk down the stems and branches to crevices in the bark of the trunk, with the time between alighting on a plant and entry into a fissure in the bark ranging from between 10 and 40 minutes (Moran & Whitham, 1990). Dispersal is important in herbivore population dynamics; by searching out new host plants herbivores may avoid crowding and food shortage (Dixon, 1972; Denno *et al*, 1991; Price, 1984). Despite their small body size, aphids are able to migrate substantial distances to locate suitable feeding sites (Loxdale *et al*, 1993).

Within just a few days of selecting a galling site, the reproductive outcome of a female galling aphid is determined by the choice of leaf. Indeed some authors have demonstrated that leaf area is correlated with greater survival and fecundity of gall-formers and leaf miners (e.g. Whitham, 1978; Tuomi *et al*, 1981; Bultman & Faeth, 1986). Other studies show that leaf quality rather than size is important for survival (Schultz, 1983; Sitch *et al*, 1988). However, the state of the leaf at the time of colonisation is critical (Aide & Londoño, 1989; Akimoto, 1998; Steinbauer, Clarke & Madden, 1998; Morrow, 1983). Expanding leaves are softer and contain higher quantities of nutrients making young leaves more palatable to herbivores. Once a galling aphid selects a suitable leaf, there is a high probability that other aphids might also find the same leaf suitable for galling. In fact, many studies have documented the occurrence of intraspecific competition in herbivores (e.g. Whitham, 1978, 1986; Quiring & McNeil, 1984; Craig *et al*, 1990), where competitive interactions commonly result in a differential fitness in survival and fecundity within a habitat.

Aphids hatch in early spring when weather conditions are most unpredictable. Temperature, rainfall and wind are the three environmental factors which have the greatest effect on aphid mortality (Hughes, 1963; Dixon, 1973; Zuniga, 1991). Temperature affects the rate of development and fecundity of colonising aphids. Raindrops knock exposed aphids off leaves and the wind disturbs the foliage causing leaves to brush together in a way which could easily dislodge an aphid. Sheltering on the underside of the leaves provides some respite from the rain, and moving to the base of the petiole, where plant structures are more rigid, provides shelter from the effects of the wind.

I predicted that a) since insect performance is correlated with host plant budburst, and b) since *Pemphigus spyrothecae* is non-migratory, *P. spyrothecae* aphids would emerge in synchrony with *Populus* x *canadensis* budburst. If aphids are adapted to individual host trees, I expected gall formation to be more successful on host trees than on conspecific trees. I tested this theory using reciprocal translocation experiments on black poplar hybrid clones taken from the *Populus* x *canadensis* trees at Attenborough Nature Reserve. I examined two opposing hypotheses: a) the small size of *Pemphigus spyrothecae* stem mothers and the short time available for gall

initiation means that fundatrices will not wander far from the tree trunk and b) stem mothers select large leaves (larger leaves are found on larger shoots, which tend to be on more distal branches) for galling (Whitham 1978, 1980). The predictions from these were that galls will be congregated on a) shoots close to the trunk; or b) shoots further away on the larger shoots.

Larval survival on expanding leaves is greater than on mature leaves (Aide & Londoño, 1989), I therefore predicted that *Pemphigus spyrothecae* fundatrices would be more successful galling on immature rather than unfurled leaves. Due to competitive interactions between fundatrices (Whitham, 1979) I expected single galls to be further developed than galls sharing a petiole. Lastly, I examined the effect of the prevailing weather conditions at poplar budburst on *Pemphigus spyrothecae* mortality rates.

4. 2 Methods

The life cycle of *Pemphigus spyrothecae* Passerini, 1860 has been described by Dunn (1966) and Foster (1990). This species is not host alternating, but spends its whole life cycle on its primary host, the black poplar *Populus nigra* and its hybrids and varieties. Eggs laid the previous year in the bark on branches or on the trunk of the host tree, hatch in spring and produce parthenogenetic stem mothers (first generation). The wingless stem mothers are very mobile, and migrate out to the newly emerged shoots, where they select a petiole for gall formation. By midsummer up to several hundred offspring are parthenogenetically produced. The first instars grow into wingless adults (virginoparae, second generation), and these in turn produce both thick-legged and normal-legged first instars. The normal-legged instars grow into winged sexuparae (third generation), that, once the galls have opened in August, migrate to the trunk, give birth to sexuals (fourth generation), which mate and produce overwintering eggs in the bark of the tree.

The hybrid black poplar (*Populus* x *canadensis*) trees used in this study are located at Attenborough Gravel Pits, Nottingham, England, a Site of Special Scientific Interest (SSSI), jointly managed by Nottinghamshire Wildlife Trust and owners Butterley Aggregates Ltd. Mature trees were used, as juvenile trees are resistant to galling and are avoided by migrants (Kearsley & Whitham, 1989). The trees grow close together along a stream near a disused car park, and hence are all subjected to similar climatic and environmental conditions. The trees were planted approximately 25 years ago.

To examine the pattern of budburst of *Populus* x *canadensis* I selected 13 poplar trees and during April recorded 20 unbiasedly selected buds daily from each tree and noted the following details: bud length and width; bud stage (classified as being closed, intermediate or open) and the distance of the bud from the main tree trunk (to the nearest 1/2 metre). Once the bud was fully open, bud length and width were unrecordable, and so the number of leaves on the bud (shoot) was counted. By mid-April stem mothers began hatching from the tree trunk and I recorded the number of stem mothers per leaf (see Plates 4.2.1 and 4.2.2); the location of the stem mothers on the shoot; the number of dead stem mothers; any predators; and the number of other

aphids. Three species of *Pemphigus* are commonly found on *Populus* x *canadensis*, but it is possible to distinguish them by appearance. Not only are they are different sizes and colours (e.g. *P. spyrothecae* is black, whereas *P. filaginis* is peach-pink), but they also form different types of galls. *P. spyrothecae* and *P. bursarius* form galls on the petiole, and *P. filaginis* initiates galls on the underside of the leaf lamina. *P. spyrothecae* is by far the most abundant.

To test whether aphid stem mothers are adapted to their particular host tree, I set up a series of translocation experiments to establish if fundatrices can initiate successful galls on conspecific trees. Hybrid poplar (*Populus* x *canadensis*) cuttings were taken from Attenborough Nature Reserve the previous year and were rooted in hydroponic baths in a greenhouse (Fig. 4.2.1). The solution used in the hydroponic baths contained:

Macronutrients 1ml/l:

24.7 g/l	$MgSO_47H_2O$	20ml/l
14.9g/l	KCl	20m/l
12.3g/l	$CaCl_22H_2O$	20m/l
6.2g/l	Na_2HPO_4	20m/l
9.6g/l	NH_4NO_3	20g/l
12g/l	Fe Citrate	1m/l

Three trees from Attenborough were selected for cloning, namely tree D, and neighbouring trees G and H. Once a strong root ball had established, the cuttings were placed in pots containing John Innes No 3 compost, and placed outside by the greenhouse. To ascertain whether *Pemphigus spyrothecae* stem mothers could form galls on other species, cuttings from the grey poplar (*Populus canescens*) were also rooted and potted up. The following spring, prior to the translocation experiments, all clones were given a sprinkling of the fertiliser Vitax Q4 and a dose (1 teaspoon/gallon water) of Phostrogen and topped up with John Innes No 3 compost.

Emerging stem mothers were collected from the three trees at Attenborough and placed in a reciprocal design on each of the clones using a fine paintbrush (see Appendices 4.3.1a-c). Tanglefoot® was smeared at the base of each clone to prevent migration onto the clone by any stray stem mother. Three days later the progress of all

aphids was recorded, and approximately two weeks into May, a final count of aphid success (gall initiation) was noted. The experiment was repeated with a second batch of aphids placed on a different set of clones 3 days after the initial translocation. Stem mothers were transferred onto the clones by tying the shoot or leaf to the clone, thus avoiding any direct contact with the aphid.

To examine petiole selection I followed the colonisation of an individual *Populus* branch by *Pemphigus spyrothecae* aphids from the day the first fundatrices were spotted (22 April) until galls were close to completion. This entailed studying each of the 20 stems on the branch every day and recording the movement of any stem mothers migrating down the branch (Fig. 4.2.2). Due to the minute size of the stem mothers (0.7mm) it was not possible to mark the nymphs. I also noted any dead aphids, aborted/rejected galling sites, and the state of gall formation, first in the degree of petiole bend, and later in the petiolar spirals. The length and width of the leaves on each shoot (a total of 115 shoots) on stems (each with between 1 and 12 shoots) were recorded on a daily basis.

To view an overall distribution pattern of aphid settlement, I randomly censused 100 galls from each of 10 *Populus x canadensis* hybrid trees during early May. The process of gall formation follows several stages, and gall development was annotated 'kink', 'bend', 'single spiral' or 'double spiral' (Fig. 4.2.3). Single and multiply-galled petioles were classified as in Fig. 4.2.4. The galled petiole was numbered and the size of the leaf measured, together with the size of biggest leaf on the shoot and the distance to the trunk. As an indicator of shoot size, the biggest leaf (length in mm) on the shoot was used. Since the average length of the biggest leaf differed among trees, these were standardised among trees. I also noted any other insects found on the shoot.

To ascertain how stem mother mortality was related to climatic conditions, I collected the following daily meteorological data for the month of April from Nottingham Weather Station: wind speed (knots) and wind direction, temperature (°C), relative humidity (%), rain fall (mm), and number of daily sun hours.

Other *Pemphigus* species were found sharing the same leaves as *P. spyrothecae* (Fig. 4.2.5). Both *P. bursarius* and *P. filaginis* are found in small numbers on these poplar trees, and although I never witnessed any direct interspecific competition, i.e. fighting as described by Whitham (1979), I would expect any aphid sharing the same leaf and/or petiole to suffer reduced fitness due to a decline in resource availability (see Plate 4.2.2). The census data revealed other insects present at the time of colonisation (Fig. 4.2.6). Large colonies of *Pterocomma populeum*, a very robust aphid, were found covering tree trunks and main branches. This aphid is densely haired and cryptically coloured, and lives on the bark of willows and poplars. *P. populeum* did not appear to affect the colonisation of *P. spyrothecae*.

Chaitophorus leucomelas, a green aphid, was first detected on the poplar buds in early April. This aphid is known to cohabit *Pemphigus* galls (Foster, 1990), but when they enter the gall remains unknown. Their distribution during *Pemphigus* hatch revealed that, on average, more *Chaitophorus* were found closer to the tree trunk (Fig. 4.2.7). They were present when *Pemphigus* stem mothers emerged later in April, and I observed them crawling about freely first on the buds and later on the young shoots. *Chaitophorus* were often seen in small groups, but whether this affected *Pemphigus* fundatrices making site selection is unclear. However, stem mothers were observed occupying the same young shoots as *Chaitophorus* aphids.

The data used in this chapter were collected during two successive spring periods, but data from each year were kept separate to examine different aspects of colonisation. Sample sizes and standard errors are included where possible in graphs showing mean values. The data were checked for normality, and where necessary, non-parametric statistics were used for analyses.

4. 3 Results

a) budburst and aphid emergence

I have already shown there was a significant difference in budburst date among the poplar trees at Attenborough Nature Reserve (see Chapter 2). Data from the 20 random shoots taken from *P. spyrothecae* hatch until gall formation, revealed that there was a significant difference among trees in the number of stem mothers per tree found each day during the colonisation period April 19th to 28th (Fig. 4.3.1). Tree K had by far the greatest number of fundatrices, whilst trees B, I, J and L all had similar numbers. Aphids on all trees appeared to be equally likely to die (Fig. 4.3.2). The poplar buds remained tightly packed until the 11th April, as seen by the sudden increase in bud width. At this point I began recording the number of leaves per bud (Fig. 4.3.3). Whitham (1978) noted that *P. betae* emergence was closely synchronised with poplar budburst, but this is not true for *P. spyrothecae* because stem mothers did not emerge until 19 April (8 days later). Aphid emergence peaked five days later, then there was a sharp decline, and a levelling off since most stem mothers had commenced gall formation (Fig. 4.3.4 and Plate 4.2.1). If aphids are adapted to the budburst of individual trees, then there should be a correlation between budburst dates and stem mother emergence on individual host trees. This seems unlikely because budburst dates were very different among trees, but stem mothers emerged on all trees within two days: there was in fact no relationship (Fig. 4.3.5). Since the sample size was low (n = 13), the power to detect a correlation was also low, it is therefore possible that there is a low positive correlation. Mortality was greatest in the first few days following hatch, when stem mothers face a vulnerable time until the gall can protect them from adverse weather conditions and potential predators (Fig. 4.3.4). Whether gall formation was a success or resulted in an abortion was independent of time of aphid hatch (Fig. 4.3.6).

Reciprocal transplants onto poplar clones of aphid from three trees tested whether aphid galling success is adapted to individual trees, a set of translocation experiments were set up (raw data see Appendices 4.3.1a-c). Aphids from tree D did not perform well on any of the clones, and only one successful gall was established (Appendix

4.3.1a). 18 stem mothers were initially placed on the various cuttings, with an outcome of 2 gall failures and 1 successful attempt. The second placement was equally unsuccessful with 9 stem mothers placed onto a cutting, but only 1 failed attempt at gall formation found. Aphids from tree G did much better (Appendix 4.3.1b): out of 21 stem mothers transferred, 11 galls were initiated and only one failed. None of the second batch of fundatrices produced any galls. 14 aphids from tree H (Appendix 4.3.1c), were also placed on various clones: 5 galls and 1 abortion were recorded in May. Again, no galls resulted from the second translocation. Fig. 4.3.7 summarises the overall outcome of the transfer experiments. Tree D aphids only produced 16.7% gall initiation (whether aborted or successful) on all clones (D, G & H). Aphids from tree G were more successful at forming galls on its own and neighbouring tree (over 80% and 60%, respectively) than on tree D, and H aphids also performed well on their own host tree and neighbouring tree G as opposed to tree D (16.7%). On the 29 April 7 aphids were placed on grey poplar (*Populus canescens*) clone 1, 8 on clone 2, and 6 aphids on clone 3. No galls were formed.

b) aphid movement

Observations from the translocation experiments on the 25th April revealed that *Pemphigus spyrothecae* fundatrices moved a great deal around the plant (Appendices 4.3.1 a-c and Fig. 4.3.8). The vast majority of such movements were towards the clone apex (86%), with downwards migration or no movement (from point of transferral) occuring at equally low frequencies (7%). The total number of movements (classified as being from bud to bud) on clones from each tree was mapped against the percentage galling attempts (of all aphids placed on the clones) (Fig. 4.3.9). Aphids placed on clones from tree G spent least time searching for suitable galling sites and were more successful at gall initiation than on clones from both tree H and D, where more aphid movement took place.

Aphid movement from the poplar tree trunk (point of aphid hatch) to the shoots was also examined. At the time when *Pemphigus spyrothecae* colonise the trees, bigger shoots (Fig. 4.3.10) containing more leaves (Fig. 4.3.11) are further from the tree trunk than smaller shoots. The data showed that although the number of galls per

shoot decreased with increased distance to the poplar tree trunk (Fig. 4.3.12), this was independent of shoot size (Fig. 4.3.13). There was no relationship between gall distance to the tree trunk and gall density per leaf (Fig. 4.3.14), indicating that aphids do not congregate on leaves at any particular distance from the tree trunk.

c) shoot colonisation

Since *Populus* x *canadensis* budburst occurs over a period of several days, the proportion of buds that are open is initially small (see Figs. 3.3.6 and 3.3.7). Therefore, a delay relative to budburst in *Pemphigus* hatch may be beneficial to aphids, reducing both search time and mortality. By the time aphid arrival peaks, not only are most buds open, but many of the pre-formed leaves are beginning to open. The basal leaves are first to unfurl on about April 14th and by April 20th, most of the remaining leaves on the shoot are unfurling. Stem mothers arriving now have an entire spectrum of leaf sizes to choose from. If leaves on shoots are divided into apical, upper middle, lower middle and basal groups, the average size of the middle leaves of shoots of *Populus* x *canadensis* is larger than both the apical and basal leaves. Previous work showed that more than 50% of stem mothers initiate their galls in the upper-middle part of the shoot. Galls generally establish on the top three leaves of the shoot (Appendix 4.3.2). There was no significant difference in position of successful or aborted galls on the shoot or in the size of these shoots (measured as the number of petioles per shoot) (Fig. 4.3.15). The census data revealed that more than 55% of fundatrices were either galling on the biggest leaf or within 10 mm of the size of the biggest leaf (Fig. 4.3.16). 24.6% of single galls and 22.2% of double galls were formed on the biggest leaf on the shoot.

The state of individual leaves at the time of selection is tabled in Appendix 4.3.2, a summary of activity on the branch I selected to follow intensively during the colonisation period. 24 stem mothers initiated successful galls on petioles with closed leaves and only one on an unfurled leaf. Six gall failures were on petioles with open leaves and two on closed: thus there was a significantly greater chance of failure on a petiole with an open leaf (Fig. 4.3.17). The four dead aphids were found either on petioles with closed leaves or on a closed bud. The latter aphid was seen positioned on

the closed bud for some time as if waiting for the bud to open, but it died before this happened. However, choosing an unsuitable site for gall formation does not always result in mortality. On 24th April a *P. spyrothecae* stem mother started galling on the 4th (basal = 1) petiole (out of six on the shoot) where the leaf had unfurled. Two days later this aphid had moved to the apical petiole, leaving a kink in the 4th petiole. Galling on the apical petiole, which had a closed leaf, was successful. This same sequence of events occurred on two other occasions (Appendix 4.3.2). Stem mothers started the galling process, but finding the petiole unsuitable, moved to a petiole with a closed leaf closer to the shoot apex. The initial rejected petiole always had an unfurled leaf.

d) gall development

Of the 1000 galls examined, 566 were single galls, 164 were pairs of double galls, there were 34 sets of triplets and 1 set of quadruple galls per petiole. 119 galls were merely kinks, 166 were bends, 463 had attained a single spiral, and 252 galls were the complete double spiral galls (Fig. 4.3.18). Single galls had finished development earlier, 34% were the completed double spiral, as opposed to 16.5% D1 and D2, whilst only 6% of the triple galls had reached this maturity. Triple 1 and triple 3 galls were both further ahead than triple 2 galls, while the quadruple galls were all still at an early stage of development. Double galls also differed in their developmental stages (Fig 4.3.19) with D2 (furthest from the leaf) often more advanced than D1. When comparing stage of development of single galls with D2 and T3 (both furthest from the leaf), 78% of singles had reached spiral galls, whereas D2 had 73% and T3 had 62% of galls in spiral form.

In the absence of a stem mother, a kink in the petiole denotes a gall failure. This can occur if the stem mother is disturbed during the initial probing either by selecting an unsuitable gall site and then moving on or by dying before the gall is formed. Fig. 4.3.20 illustrates how gall failure was spread across the three gall categories. There were fewest failures of single galls, D2 galls were intermediate, and all three triple galls had high failure rates.

e) aphid mortality due to weather

Adverse weather conditions can seriously take its toll on newly emerging insects. Measurements taken from Nottingham weather station revealed that the daily temperature ranged between 9.1-16.1 °C, with an average temperature of 11.7 °C. Relative humidity ranged between 61-91 %, and was an average of 74.6 %, whilst the daily wind speed was between 5-15 knots, averaging 9 knots. To explore if there was any relationship between the percentage aphid mortality and the prevailing weather conditions, a multiple regression was performed, although with a sample size of only n = 10 days, this can only suggest possibilities rather than test critically for these relationships. Wind speed, temperature, number of sun hours, relative humidity and amount of rain fall were used to calculate a multiple R value of 0.95 ($R^2 = 0.91$, n = 10 (days), $p < 0.05$). However, using five variables to predict ten points always produces high R^2 values. There is also a problem with multicollinearity because the variables are highly intercorrelated. In order to test for relationships, partial correlations were calculated to control for the effects of the other variables. Although not statistically significant (as expected for such small sample sizes), as both wind speed (Fig. 4.3.21) and temperature (Fig. 4.3.22) increased, the percentage dead *Pemphigus spyrothecae* stem mothers appeared to increase, but decreased as relative humidity increased (Fig. 4.3.23). The amount of rain (Fig. 4.3.24) or the number of daily sunshine hours (Fig. 4.3.25) did not appear to affect the survival of *Pemphigus* nymphs.

4. 4 Discussion

Among-tree variation in budburst dates is common in many tree species, e.g. elms (Komatsu & Akimoto, 1995; Akimoto, 1998), oaks (Hunter, 1992; Van Dongen *et al*, 1996) including the poplars in this study. Synchrony of host tree budburst and insect emergence is important in many herbivore systems, for instance aphid nymphs on individual host trees have an optimal hatching time, with lowered performance in earlier and later hatch than at budburst time (Akimoto & Yamaguchi, 1994), and caterpillar density is correlated with budburst in oaks that leaf early (Hunter, 1992). In the study year *P. spyrothecae* stem mothers timed their emergence to poplar budburst, and although I did not find evidence of any relationship between timing of *Pemphigus* hatch and galling success, asynchrony between egg hatch and budburst is a likely cause of high mortality rates of newly emerged insects.

Egg-hatching time and gall formation of some aphids such as *Kaltenbachiella japonica* on *Ulmus davidiana* var. *japonica* (Komatsu & Akimoto, 1995) and *Tetraneura yezoensis* also on *Ulmus* trees (Akimoto, 1990) are positively correlated with mean budburst time on individual trees, suggesting that aphids are genetically adapted to the budburst phenologies of their host tree. *Pemphigus spyrothecae* is non-migratory like *Kaltenbachiella japonica*, but emergence did not appear to be adapted to individual poplar trees. Stem mothers hatched when the poplar trees had been opening buds for over a week and all trees were more or less at the same stage of flush. However, having only a power of 38%, the test used may not be powerful enough to detect any relationship. 50 trees would give a power of 90% (using a 1-tailed test), and would more accurately pinpoint any synchrony between budburst and aphid hatch: however, such numbers were not possible in this study. Adaptation of herbivores to individual host plants may happen if plants are long lived and have traits different among individuals, and if herbivores are so sedentary that there is little gene flow between populations on individual plants (Edmunds & Alstad, 1978).

Habitat selection operates such that insect migrants should sense differences correlated with the budburst time of individual trees, and select those trees to which their offspring will be adapted (Hedrick, 1986). Variation among trees in variables

such as average egg-hatching time may be genetic, indicating a restricted gene flow between aphid populations on different trees (Komatsu & Akimoto, 1995). To ascertain how closely *Pemphigus spyrothecae* aphids are associated with individual host trees, I performed a set of reciprocal translocation experiments. Although the sexuparae are winged they are unlikely to migrate far, so there is likely to be some gene flow between populations on trees G and H, and this would account for the ability to successfully initiate galls on the non-host tree. Tree G also had the least aphid movement and the highest gall success, indicating that this is a particularly susceptible host tree. Further translocation experiments using larger sample sizes and/or genetic fingerprinting would confirm any genetic relationship between the two populations. Akimoto's (1998) study using nymphal transplantation experiments suggests that early hatched nymphs are more successful at galling on early leafing trees than on late leafing trees and visa versa. Trees G and H both had all the buds open closer to the arrival of the stem mother than tree D (see Table 3.3.1).

P. spyrothecae aphids are specific to black poplars, and are likely to behave in a similar way irrespective of host plant site or environmental conditions. However, other insect groups have more than one species of host plant, and are known to respond to these in very different ways. For instance, although hatching of the winter moth *Operophtera brumata* coincides with the new growth of common heather, *Calluna vulgaris*, delaying egg hatch by up to one month did not cause any negative effect on larval performance, despite the decrease in nitrogen content of heather during this period, i.e. the winter moth maintained larval development on heather despite the decreasing quality of food resource, proving that synchrony of budburst and egg hatch is of little consequence on this host (Watt & McFarlane, 1991). A tight hatch-budburst synchrony would perhaps be disadvantageous in harsh environmental conditions and widely ranging altitudes of the moorlands on which the heather grows. However, *O. brumata* is more sensitive to budburst in oak trees (Feeny, 1970). Egg hatch is closely synchronised with individual trees, partially promoted by the female's brachyptery and thus low dispersal ability. Males, though winged and capable of flying to other trees, also show limited dispersal, and this may increase egg hatch synchronisation and expected fitness (Van Dongen *et al*, 1996). Although some *P. spyrothecae* winged adults probably leave the host plant in search of new sites in the

autumn, most are apt to stay close to their birth tree, and so fundatrices are unlikely to select an inappropriate host plant in spring. As expected, *P. spyrothecae* did not initiate galls on *Populus canescens*. Some migrant aphids are not always able to locate their true host and the consequences of landing on the wrong host results in the fundatrices, hatching the following year, being unable to initiate galls (Wool *et al*, 1994; Burstein & Wool, 1993).

Since the overwintering *P. spyrothecae* eggs are laid on the trunk of the poplar tree, hatching fundatrices must migrate some distance to reach suitable petioles for galling. Aphid movements are hindered by sticky exudate on young buds and small epidermal hairs covering the petiole and leaf (see Plate 4.2.1). The hairs do not stop the aphids from probing, but slow down the velocity at which the aphid moves. The maximum distance a stem mother on the woody part of a shoot can travel is approximately 2.7mm/s, compared to 0.7mm/s on the hairy petiole (Beyer, 1993). Despite Whitham's (1978, 1980) findings that *Pemphigus betae* performed better on large leaves, *P. spyrothecae* aphids were found equally spaced across the tree, even though shoots are larger further from the tree trunk, which suggests a trade-off between leaf size and the effort and risk of movement. Colonisation of the poplar branch (see Fig. 4.2.1) that I followed from *Pemphigus* hatch to galling, indicates that stem mothers do not colonise in 'waves' from the trunk to the distal shoots. On the first day of emergence (22 April) two stem mothers were seen on stems 1 and 18, and within a couple of days galling was in progress on stems 1, 2, 3, 6, 7, 19 and 20. Stem mothers also occupied stems 9, 12 and 18. On the poplar clones, stem mothers moved acropetally, indicating that they are probably committed to one branch and are unlikely to visit more than a small proportion of potential leaves. In order to estimate habitat quality the aphid must sample at least a few of the many possible habitats on offer. However, the aphid may not settle in the habitat most suitable due to the low chance of this habitat being found. In this instance the Ideal Free Distribution model of habitat selection is unlikely to reflect accurately the habitat selection process in *Pemphigus* aphids.

The importance of leaf state at time of colonisation has readily been shown in this study. Herbivores hatching or selecting oviposition sites on expanding leaves are generally more successful than those on fully expanded leaves (Aide & Londoño,

1989; Steinbauer, Clarke & Madden, 1998). Mature sclerophyllous leaves are tough and have low nutrient and nitrogen contents, whereas immature and expanding leaves are softer and contain higher quantities of nitrogen, making them more palatable for most herbivores (Morrow, 1983). Some chemical compounds, for instance essential oils, total phenols, and condensed tannins do not exhibit any consistent pattern of change from young to old leaves, but changes in nitrogen content and leaf toughness most likely explain beetle preference for young leaves (Boland *et al*, 1991). The vast majority of *P. spyrothecae* stem mothers selected petioles of immature leaves. Aphids initiating galls on petioles with open leaves often then rejected these petioles in favour of others bearing a closed leaf closer to the shoot apex (see also Fig. 4.3.15: failed galls closer to basal leaf on shoot). However, moving site is a risky strategy; 61% of *Pemphigus betae* aphids that abandoned a leaf and moved to another died in the attempt, while only 22% of those remaining in the original position died (Whitham, 1978). Only one successful gall was initiated on an expanded leaf, and 6 out of 8 gall failures occurred on unfurled leaves, indicating that unfurled leaves are too mature to be colonised. *Pemphigus populicaulis* aphids also gall on immature, not yet unfurled, leaves of eastern cottonwood, *Populus deltoides* (Rhomberg, 1984). Emergence of *Pemphigus* stem mothers appears to be timed so that, due to the sequential nature of leaf development from base to tip, basal leaves on the shoot are already too mature to colonise with the more distal leaves, destined to be larger, still immature and susceptible to galling. Even if leaves were selected at random from those available, this would account for the observed distribution of galls. Although Whitham (1980) suggests that the observed pattern of gall formation is a result of non-randomness in the aphids choice of leaves, it may be explained by the non-randomness in the availability of susceptible leaves. Aphids would, therefore, be expected to settle on leaves that will become the largest on the shoot. If emergence is timed when the larger leaves are immature, the proportion of buds open at this time approaches 100% allowing the aphid more choice to select open buds. I have observed aphids settling on closed buds, only to find them dead later.

Growth of plant cells may be described as an 'irreversible increase in volume with time'. This increase results primarily from the uptake of water into the vacuoles, which contain concentrated cell sap. The characteristic structure of the plant cell

allows this type of extension growth in volume; the protoplast together with the extensible cell wall forms an osmo-mechanical system which develops a hydrostatic pressure (turgor) on the basis of the difference in osmotic potential between the vacuolar solution and the solution in the cell wall. Turgor indirectly promotes a plastic expansion of the cell wall by water uptake, enlarging the cells 10-100 times their original volume (Mohr & Schopfer, 1994). During budburst leaf primordia change to fast extension growth by taking up water and using storage materials. If *Populus* x *canadensis* petioles bearing closed or unfurled leaves differ in their tugor pressure (perhaps closed leaves have a higher initial rate to enable unfurling), *P. spyrothecae* aphids may use tugor as a cue to determine which leaves are suitable for gall formation. Analysis of *Xanthium strumarium* leaves (similar shape to poplar) shows that different parts of the lamina have different growth rates dependent on the distance to the tip and the age of the leaf (Mohr & Schopfer, 1994). Growth rate is slowest at the tip and greatest at the base of the leaf, causing changes (mostly caused by differential growth of leaf cells) in the leaf shape during leaf development. This may explain the mechanism by which *P. betae* showed such a strong preference for the basal position on the *Populus angustifolia* leaf, a location producing 65% more offspring than distal sites (Whitham, 1978).

Aphid density on the poplar petioles had a strong effect on gall development with single galls finishing development sooner than both double galls and triple galls. The lack of any intraspecific competition may enable the single aphid to 'get on with the job'. The second of the double galls (furthest from the leaf) was also more advanced than the first gall. Unfortunately, the branch on which I observed aphid settling had no double galls, so I am unable to verify whether D2 (furthest from the leaf) is completed sooner due to the stem mother arriving earlier than the stem mother of D1. However, assuming there are no biochemical or other factors influencing development rates of galls on shared petioles, this seems a plausible explanation.

Emergence of *Pemphigus spyrothecae* occurs in spring when weather conditions are very variable. Wind, temperature and rain can devastate populations of hatching insects (Dixon, 1973; Hughes, 1963; Zuniga, 1991). In some years, early emergence of the pear thrips, *Taeniothrips inconsequens*, was linked to short spells of warm

weather, while in other years, cold weather and snow cover delayed initial hatch (Teulon, Leskey & Cameron,1998). Temperature and photoperiod have been shown to influence egg development (Hill & Hodkinson, 1996), and whereas some authors suggest rain causes an increase in insect mortality (e.g. Dhalival & Singhe, 1975), others (Araya & Fereres, 1991) state that rain in itself does not cause mortality, but wetness of aphids and leaves decrease aphid movement along leaf surfaces (Harrington & Cheng, 1984), allowing wind (Hughes, 1963) to displace aphids from plants. Figs. 4.3.22-26 illustrate the prevailing weather conditions at *P. spyrothecae* hatch. Since weather variables are highly intercorrelated, it was difficult to determine a single factor as being the major contributor to aphid mortality. Wind batters the poplar leaves, and aphids become dislodged and fall to the ground. It appears from the data that fundatrices survive better in cooler temperatures, with higher relative humidity and little or no wind. Stem mothers may require moist conditions to enable them to migrate successfully out to the petioles.

During my translocation experiments there was a high rate of *Pemphigus* stem mother mortality. The cause of this mortality may lie in the technique in which aphids were transferred from the trees at Attenborough to the clones in the University greenhouse. Before transferral most of the aphids were probing (seen through a magnifying lens) with their stylets embedded in the plant tissue. Using a paintbrush, even a very fine-haired one, to dislodge the aphid may have damaged the stylet. Even though the aphids once transferred, appeared to behave normally, any damage to the stylet may inhibit active probing. Aphids are reluctant to withdraw their stylet (perhaps reaching the sieve tubes is both a timely and costly process) even when disturbed at close proximity. If a paintbrush is placed close to a probing aphid, in order to avoid the offensive intrusion, the aphid will turn up to 360° without withdrawing the stylet (Beyer, 1993).

If stem mothers already forming galls are transferred to another petiole, they will usually die. Transfer of aphids must be early, before gall initiation, or at most when the gall is at the kink stage of formation. *P. betae* are also reluctant to move once gall formation has commenced (Whitham, 1978). In tests, 87% of stem mothers successfully moved site prior to gall initiation, whereas only 57% would move once

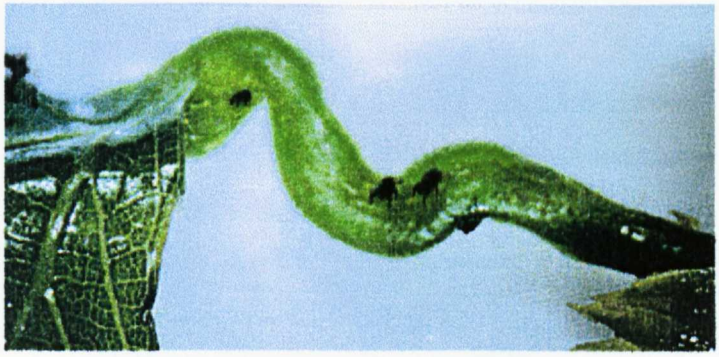
rudimentary galls had formed. A physiological change or cost must prohibit further movement. In the first part of the transferal experiment, aphids were translocated using a small paintbrush. As this might have caused damage to the aphids, in the second test I tied the whole leaf to the clone with a piece of string, so aphids could move onto the clone of their own accord. However, at this point in time most stem mothers had already started galling on trees at Attenborough.

To summarise, budburst date differed among the *Populus* x *canadensis* trees, whereas all the *Pemphigus spyrothecae* stem mothers arrived within two days, and although I was not able to prove statistically that aphids are adapted to individual host trees, budburst plays a vital role in population distribution. Within host trees, the most important factor in deciding gall location on shoots is the availability of petioles bearing closed leaves at the time of *P. spyrothecae* colonisation (Fig. 4.3.17).

Plate 4. 2. 1

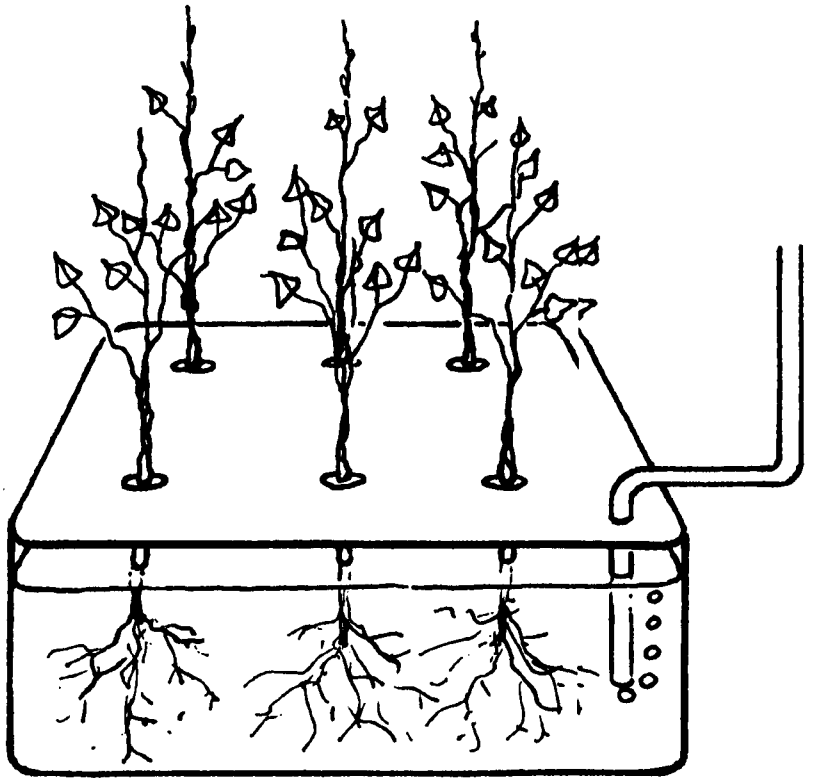
Pemphigus spyrothecae stem mother in the process of gall formation. The stem mother is only a few millimetres long, but exerts an effect powerful enough to cause the *Populus* x *canadensis* petiole to bend and eventually turn a section into a double spiral. While the aphid produces offspring, she is protected inside the gall during the coming months.

Plate 4. 2. 2



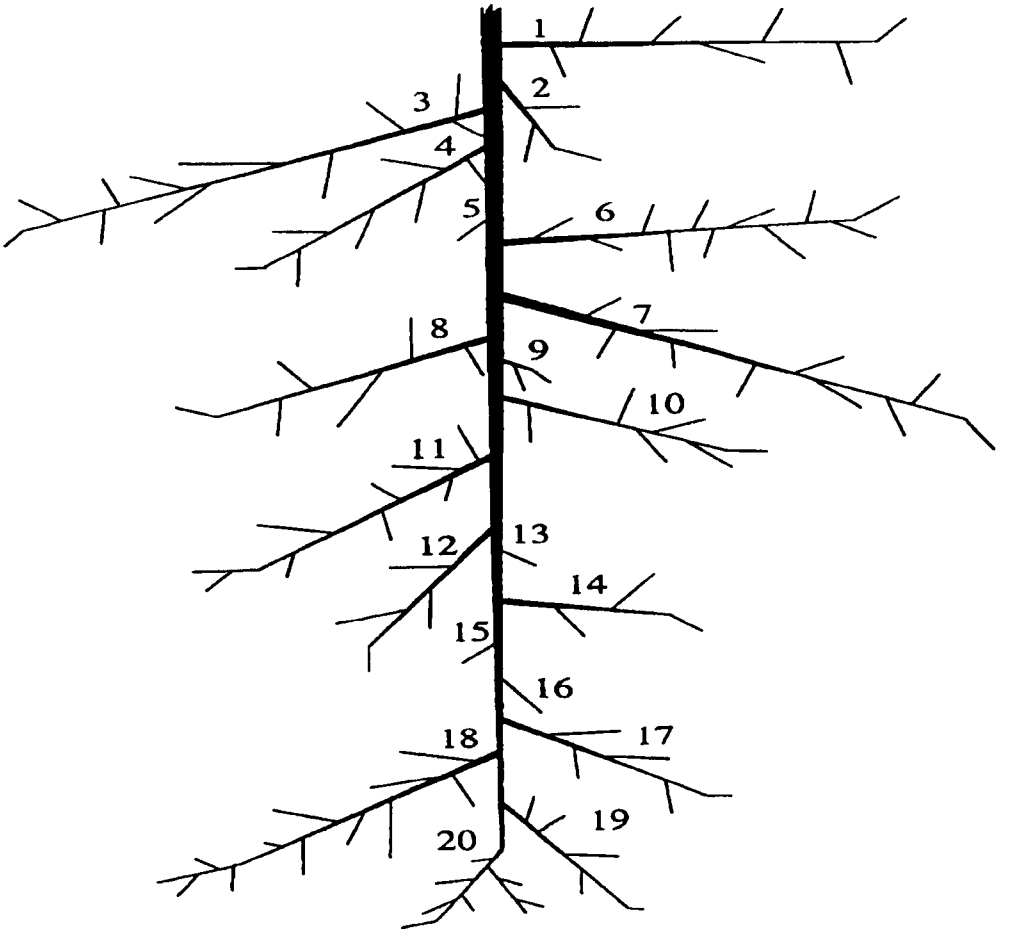
Four *Pemphigus spyrothecae* stem mothers compete for galling sites on a *Populus* x *canadensis* petiole. Interactions between fundatrices can result in death, not only as a result of falling off the petiole, but also because the time for gall initiation is short and displaced aphids must search for alternative sites.

Figure 4. 2. 1



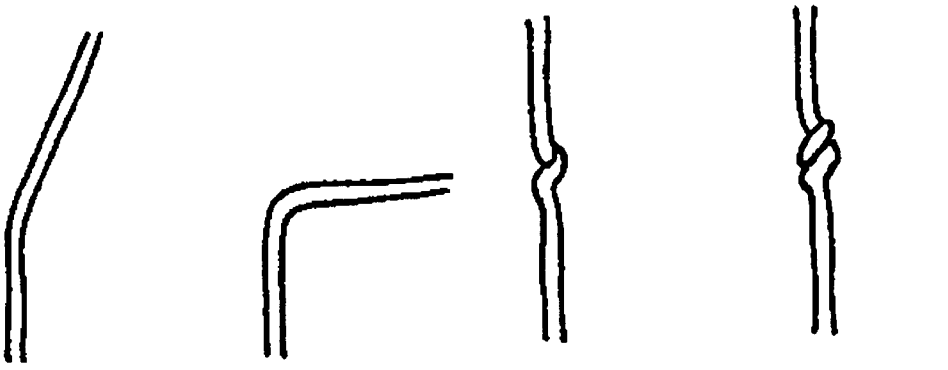
Hydroponic bath with *Populus* x *canadensis* and *Populus canescens* cuttings. This technique is used to root cuttings. Air is pumped in to the container to oxygenate the water which contains a nutrient solution (see Methods).

Figure 4. 2. 2



Populus x *canadensis* branch showing the 20 stems containing between 1 and 12 shoots on each stem.

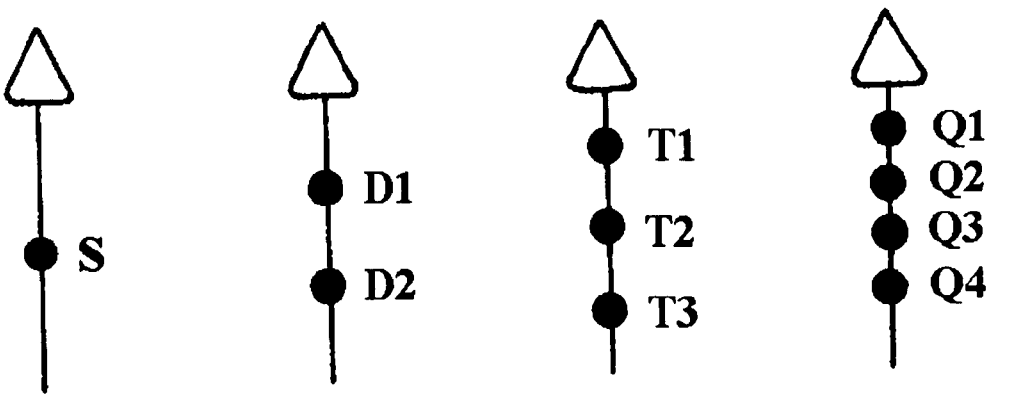
Figure 4. 2. 3



Kink Bend Single spiral Double spiral

Stages of gall formation on the petiole

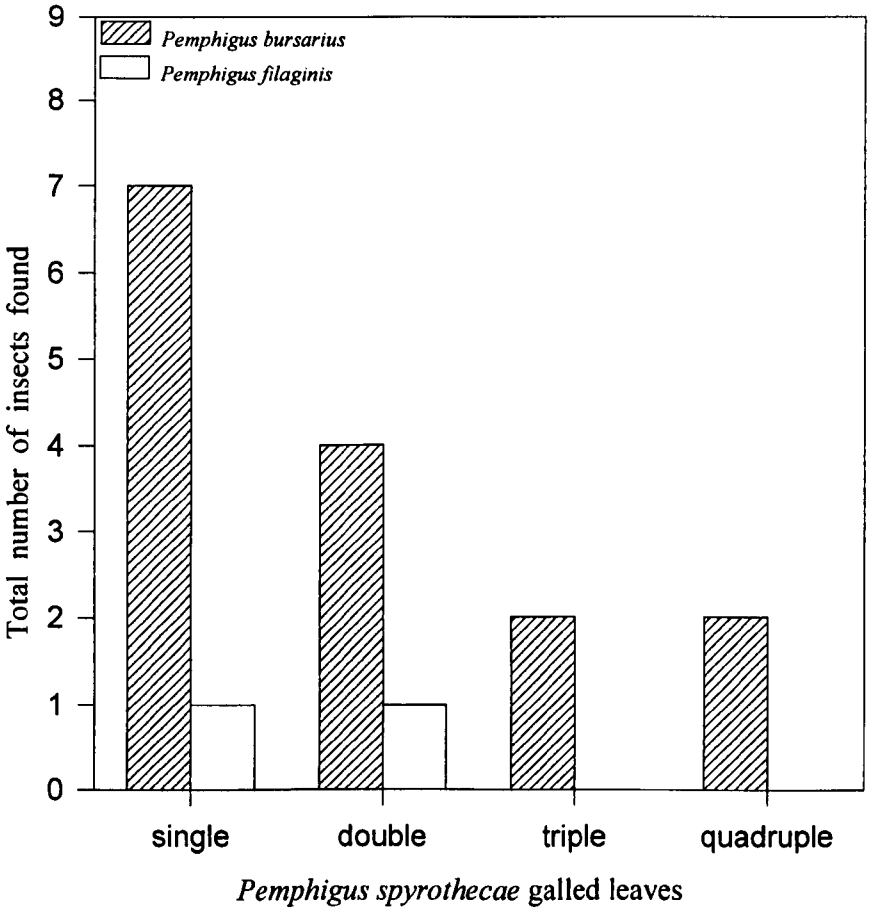
Figure 4. 2. 4



Gall annotation for number of galls per leaf

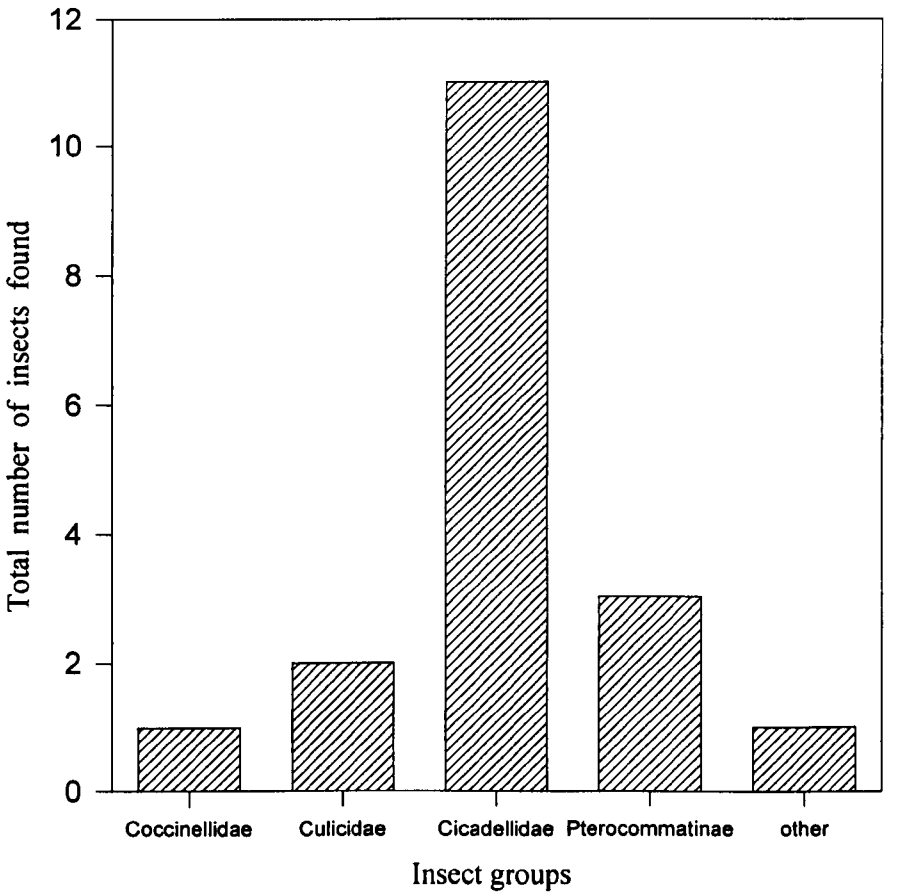
Figure 4. 2. 5

Other *Pemphigus spp.* on poplar galled leaves



Although small in numbers both *P. bursarius* and *P. filaginis* were found to initiate galls on the same petioles/leaves as *P. spyrothecae*.

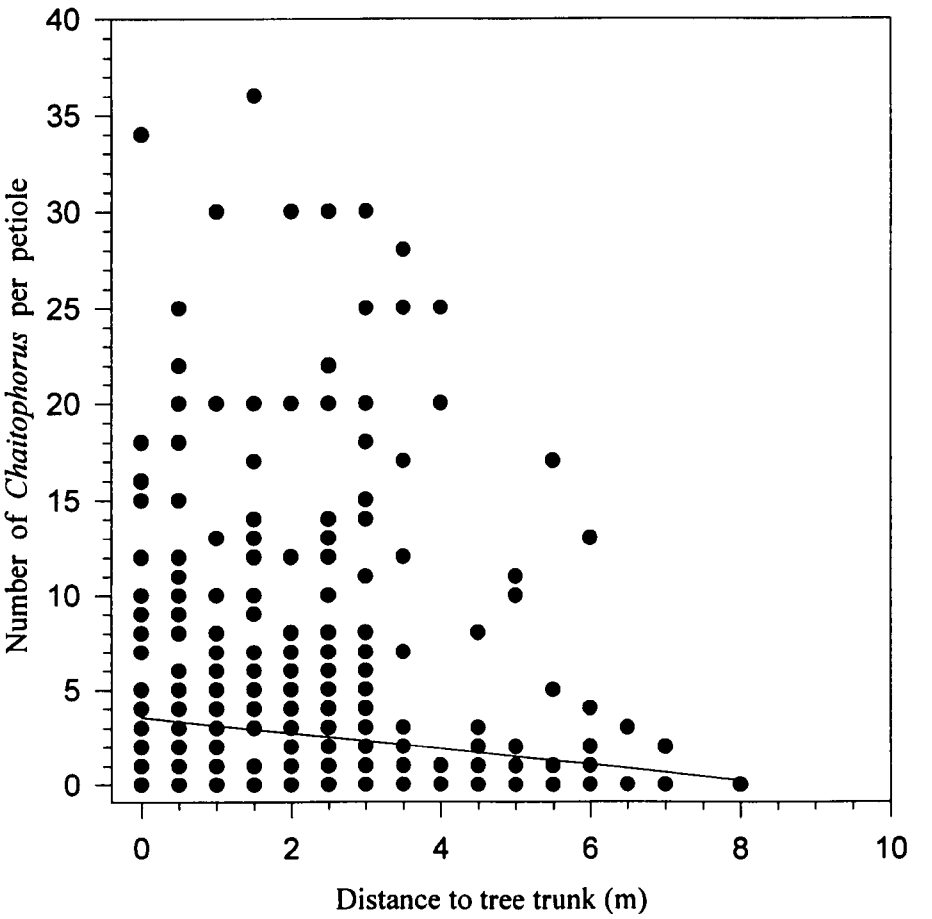
Figure 4. 2. 6



These insect groups are present at the time of *Pemphigus spyrothecae* stem mother emergence, and with the exception of ladybirds, do not appear to affect aphid colonisation.

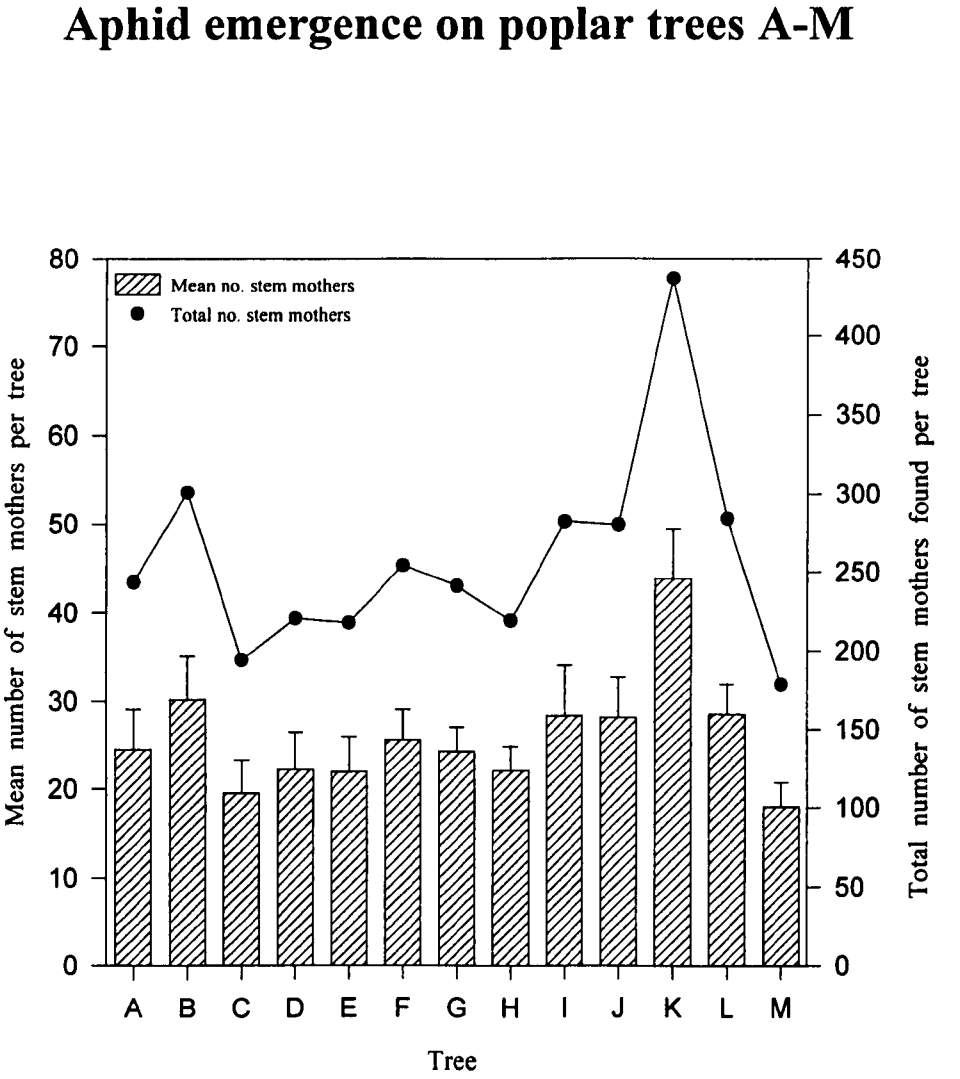
Figure 4. 2. 7

Chaitophorous leucomelas distribution from the tree trunk



At the time of *Pemphigus* emergence, *Chaitophorus* aphids did not appear to migrate far from the poplar tree trunk ($r^2 = 0.012$, $n = 763$, $p < 0.01$).

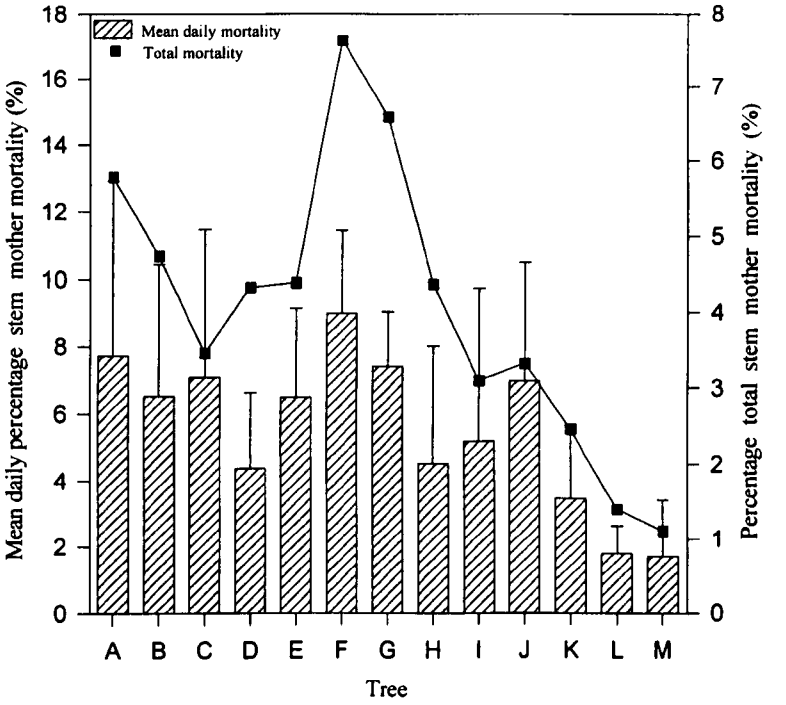
Figure 4. 3. 1



This graph illustrates the mean number of *P. spyrothecae* found each day on trees A-M over a period of 10 days (19-28 April), together with the total number of colonising stem mothers. There were significant differences among trees in the number of aphids found each day ($F_{12,129} = 2.41$, $p < 0.01$).

Figure 4. 3. 2

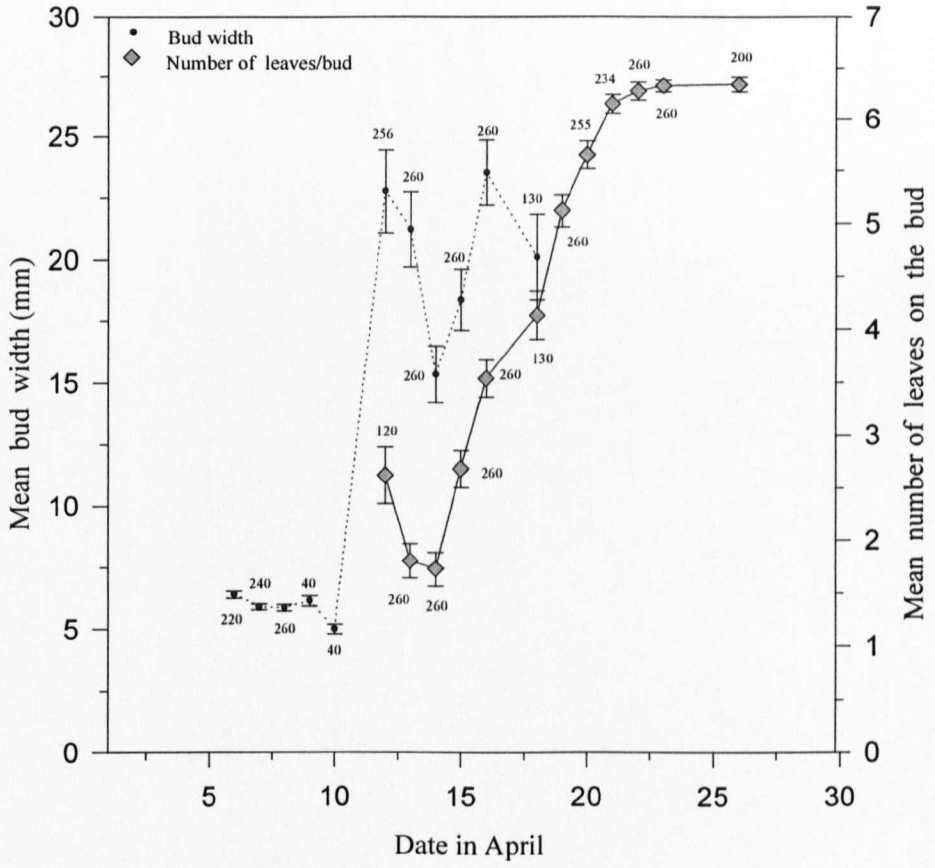
Stem mother mortality at colonisation



The mean daily percentage *P. spyrothecae* mortality ranged from nearly 2% on tree M to about 9% on tree F during the colonisation period (19-28 April). There were no significant differences among trees (Kruskal-Wallis $H_{12,123} = 15.19$, n.s.).

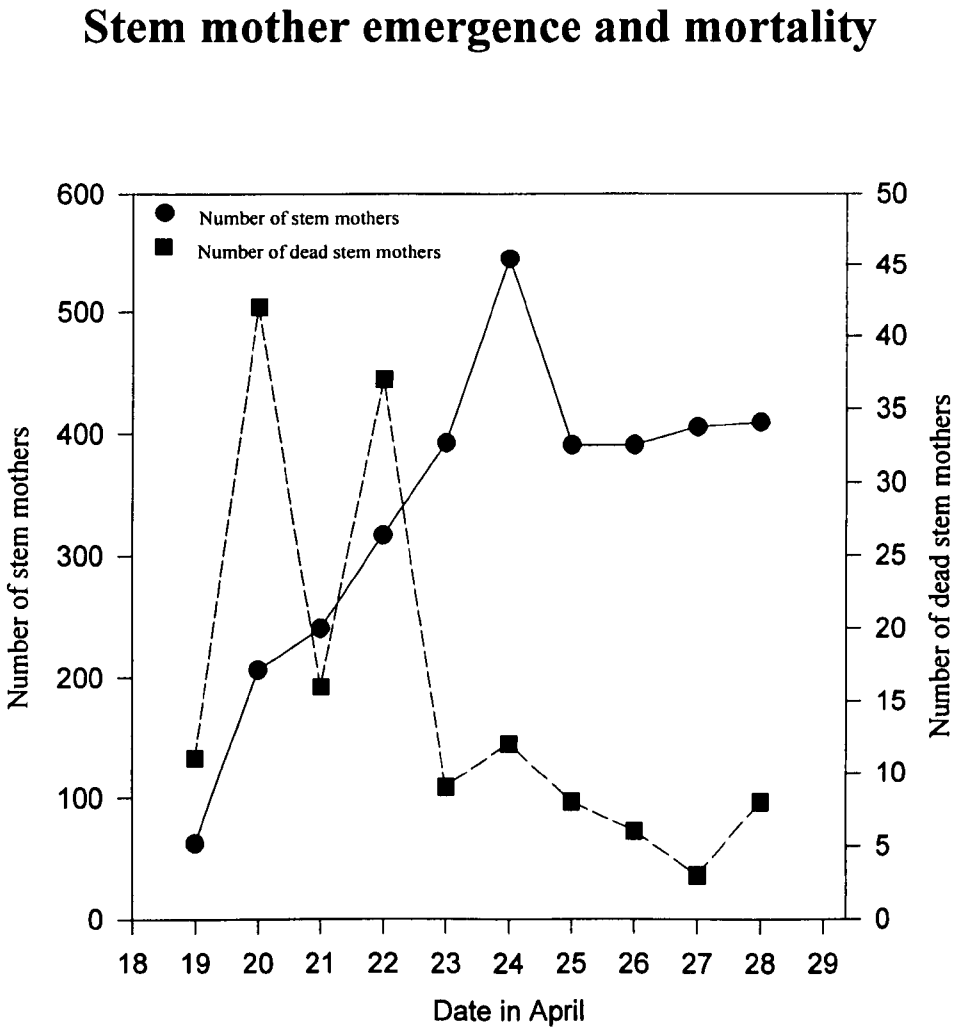
Figure 4. 3. 3

Mean bud width and number of leaves per bud



Change in average poplar bud width and number of leaves per bud during budburst in April.

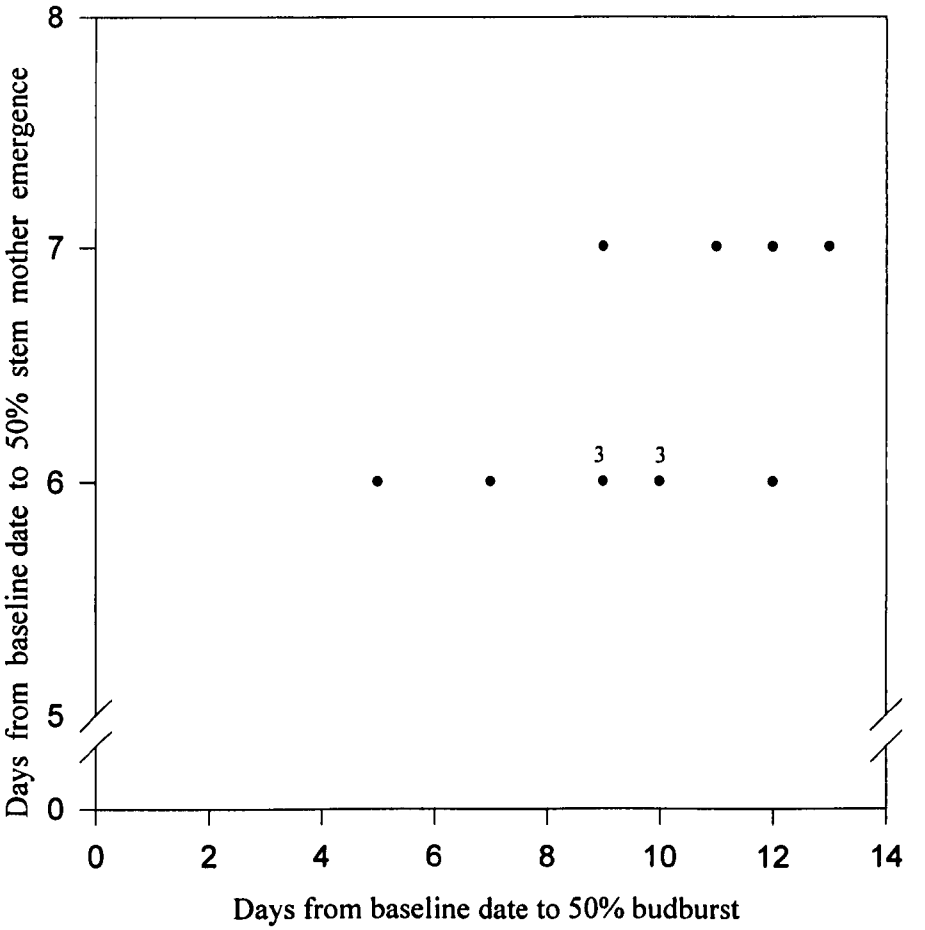
Figure 4. 3. 4



Number of hatching *P. spyrothecae* fundatrices and subsequent mortality during April.

Figure 4. 3. 5

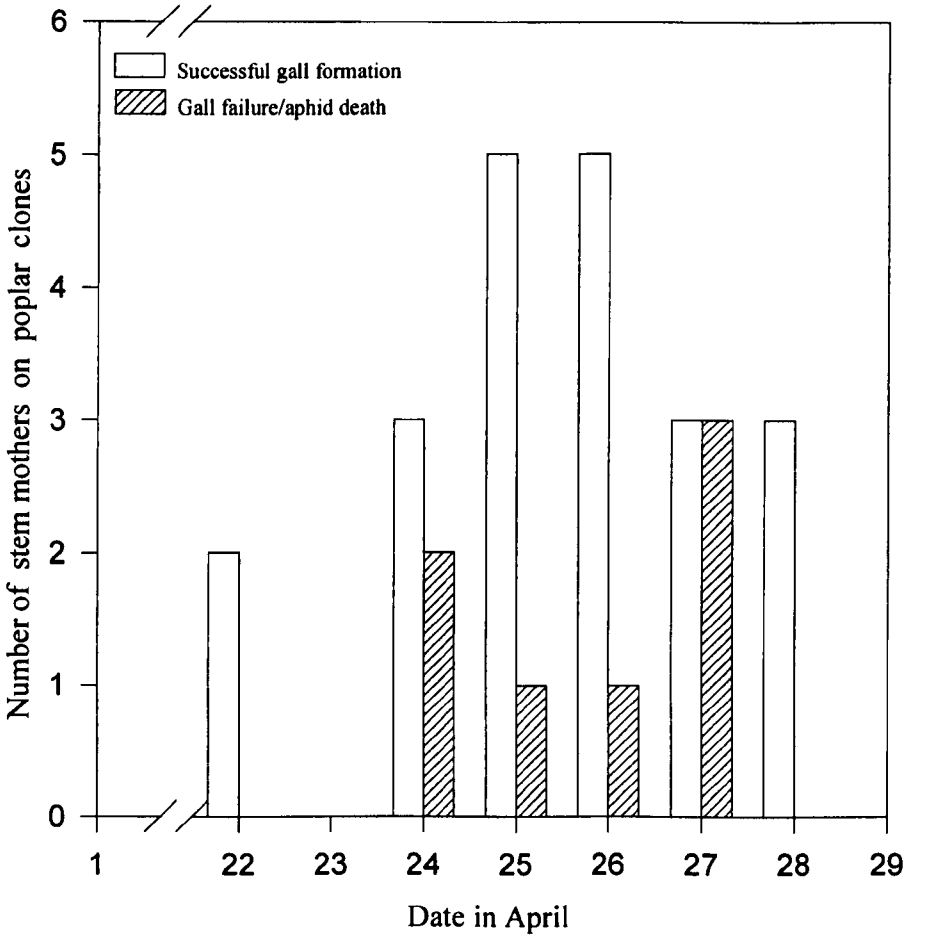
Micro-adaptation of aphid at poplar budburst



April 6th was used as a baseline date when no buds were open on trees A-M and a second point in time when 50% of all buds were open on individual trees was then calculated. This approach was also carried out on stem mother emergence using April 18th as a baseline date. There was no relationship ($r^2 = 0.014$, n = 13, n.s.).

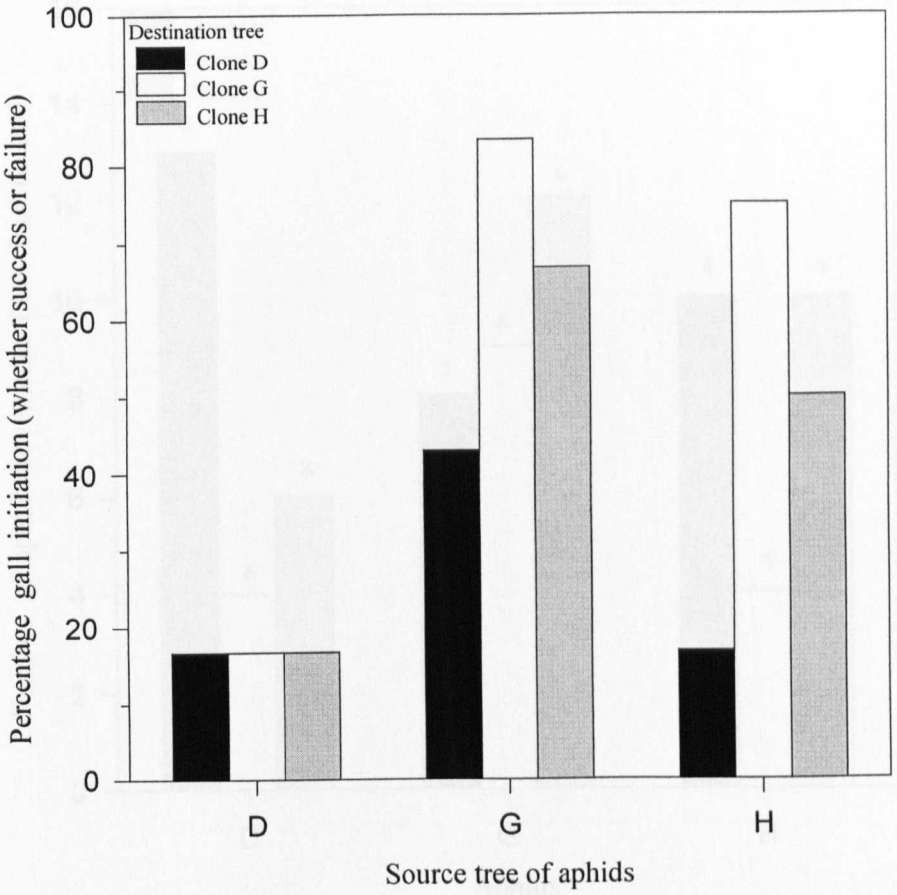
Figure 4. 3. 6

Timing of aphid emergence and subsequent galling success



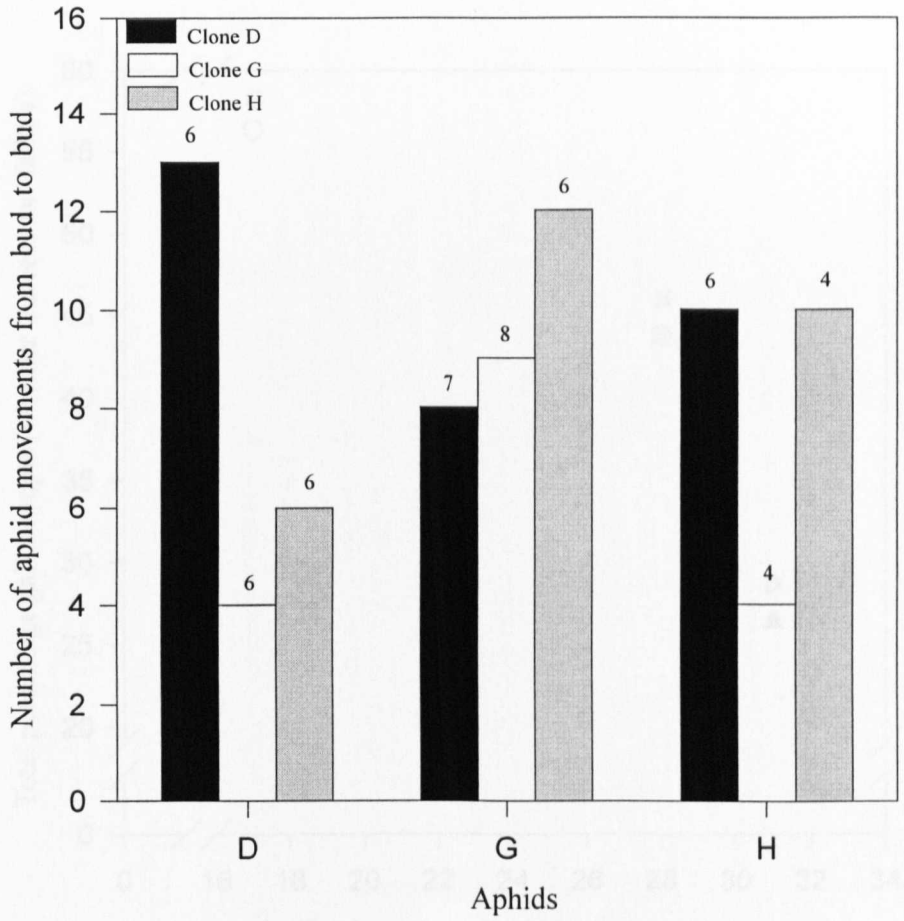
Successful *P. spyrothecae* galls were not initiated earlier in April than failures (Mann Whitney U = 70.5, n = 27, n.s.).

Figure 4. 3. 7



Summary of *P. spyrothecae* stem mother gall formation in reciprocal translocation experiments. Aphids from tree D did not perform well on any clone, whereas stem mothers from both poplar trees G and H readily initiated galls on their own and neighbouring trees.

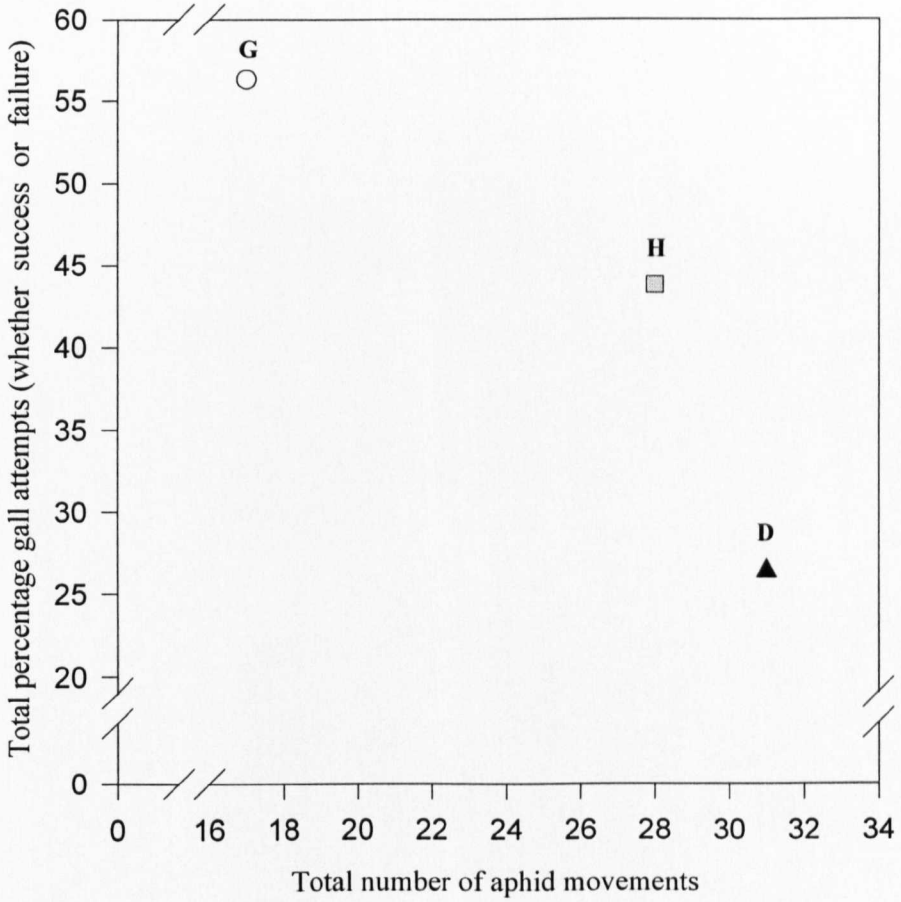
Figure 4. 3. 8



Frequency of stem mother movements on poplar clones. One movement was quantified as being from bud to bud, e.g. movement from bud 5 to bud 2 was recorded as 3. The data do not represent a continuous movement pattern as recordings were only taken on three occasions. However, this does indicate that aphids move around the clone prior to site selection. The figure above each column notes the number of stem mothers placed on those clones.

Figure 4. 3. 9

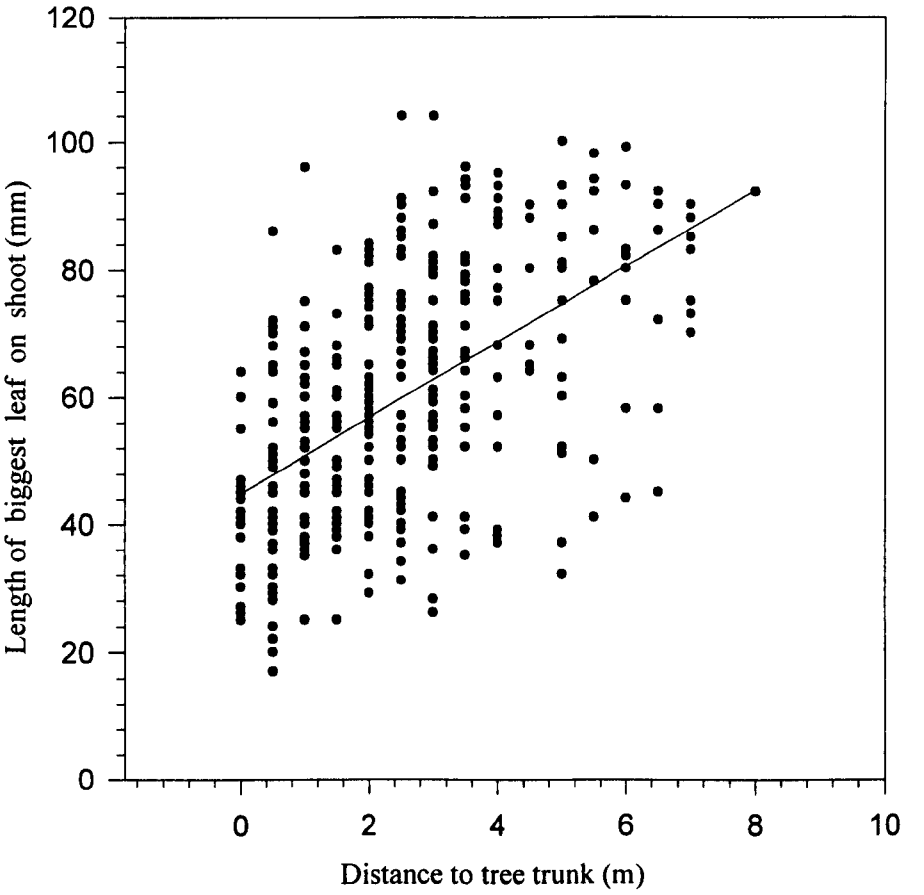
Aphid movements and gall formation



This graph illustrates the total recorded movements of all aphids (irrespective of origin) on clones from the poplar trees D, G and H against the rate of gall initiation. Stem mothers placed on clones from tree G had the fewest movements, but the highest percentage attempts at galling. In contrast, aphids on tree D clones moved about the most and had least galling attempts. By eye, a linear relationship is possible, but as the sample size is low (n = 3), more data points are required before this can be proved statistically.

Figure 4. 3. 10

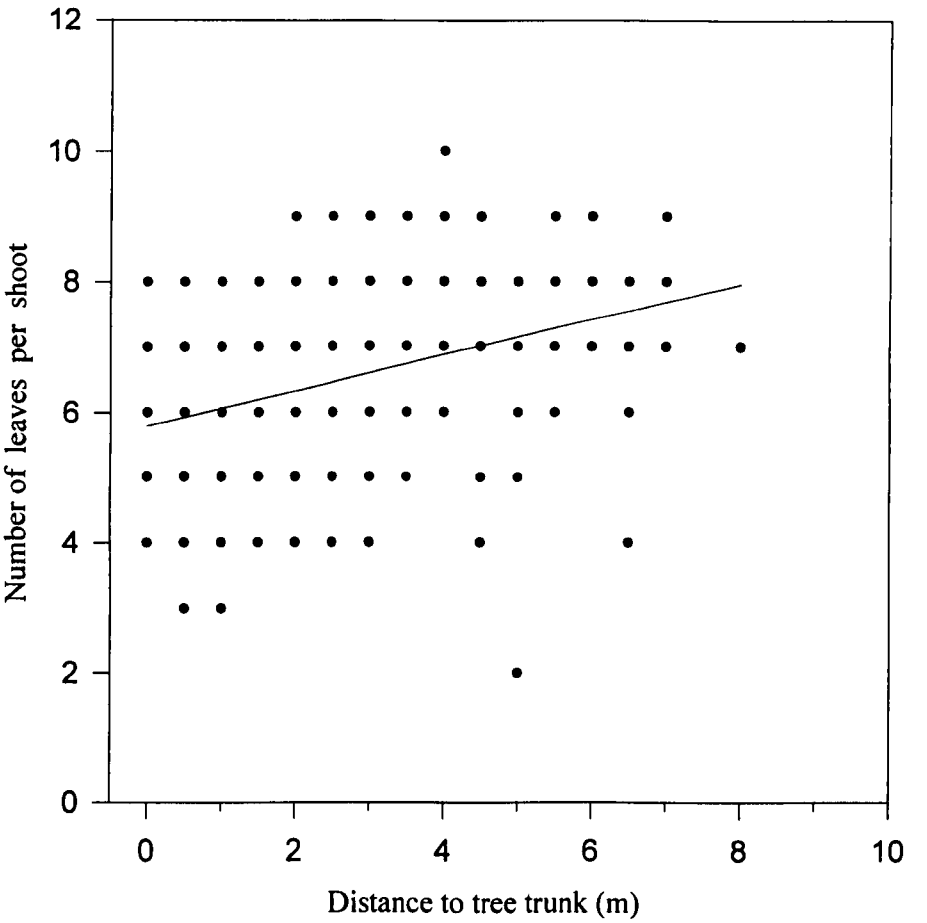
Poplar shoot size and distance to tree trunk



Shoots further from the tree trunk were bigger ($r^2 = 0.31$, $p << 0.001$, $n = 383$) (biggest leaf on the shoot being an indicator of shoot size).

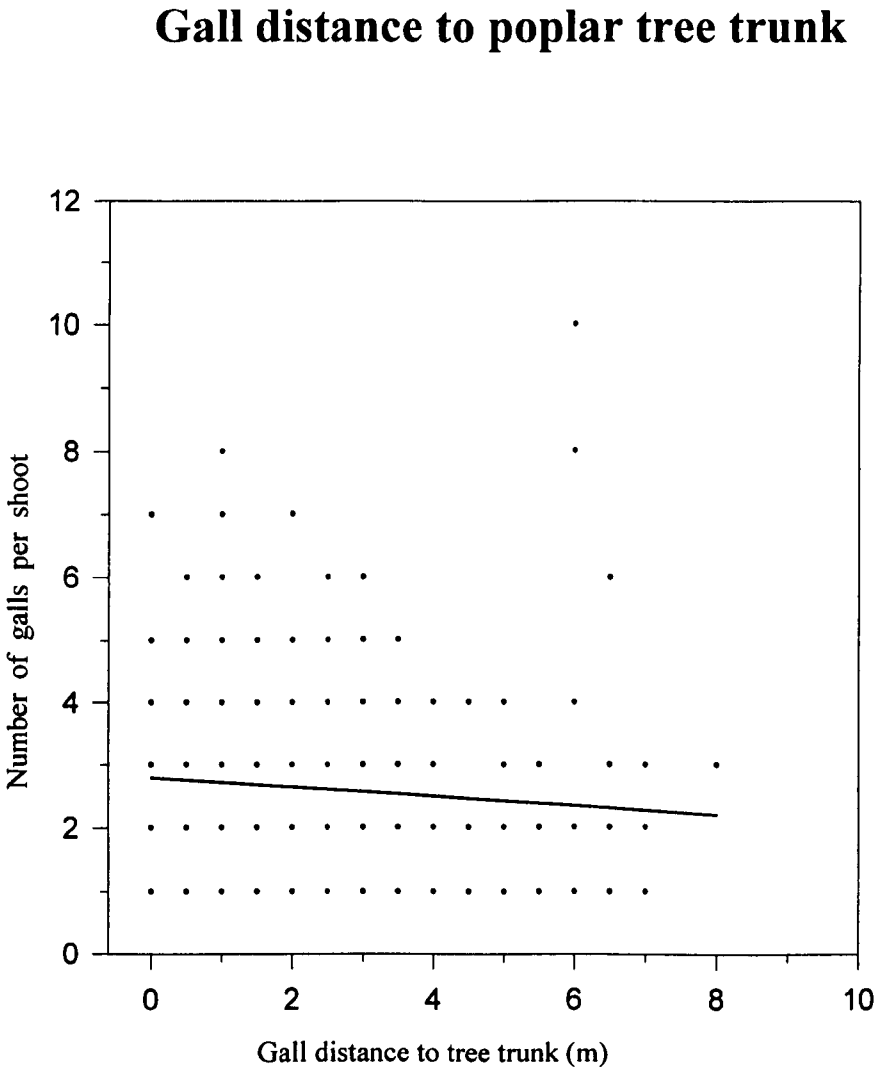
Figure 4. 3. 11

Number of leaves on poplar shoots at different distances from the tree trunk



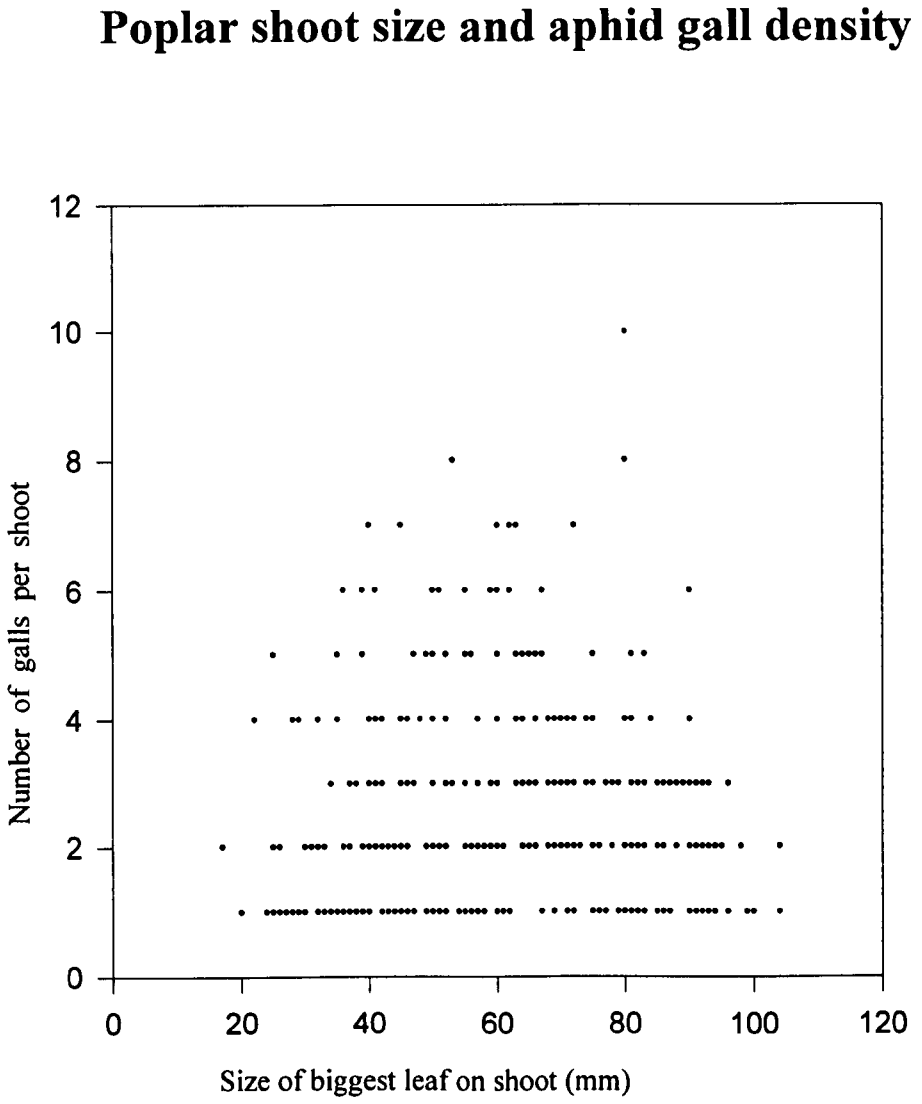
Shoots further from the tree trunk contained more leaves ($r^2 = 0.138$, $p << 0.001$, $n = 383$).

Figure 4. 3. 12



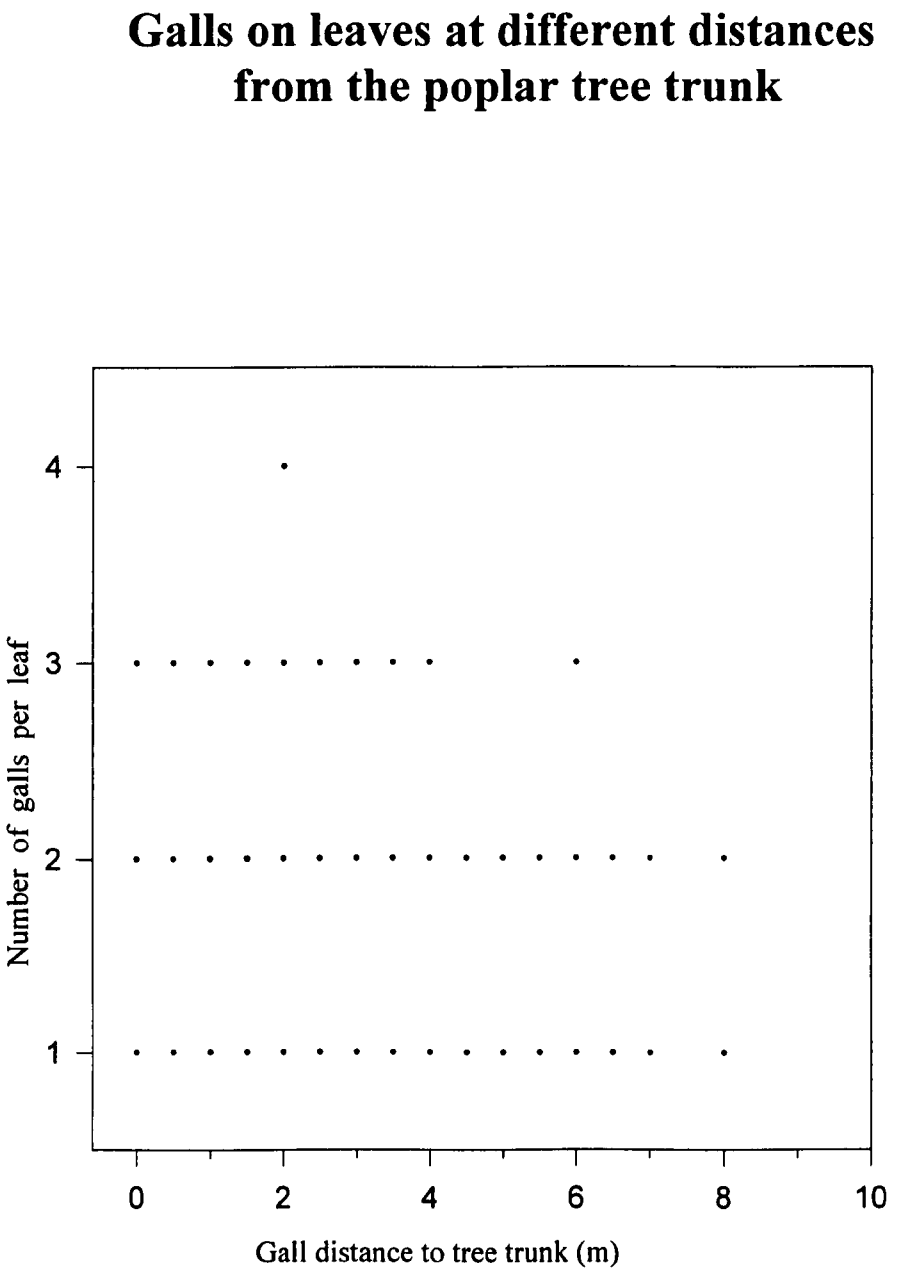
The number of *Pemphigus spyrothecae* galls per shoot tended to decrease further from the tree trunk ($r^2 = 0.02$, $p < 0.05$, $n = 381$), indicating that galls tend to congregate closer to the tree trunk.

Figure 4. 3. 13



At the time of budburst *P. spyrothecae* fundatrices did not appear to congregate on the largest poplar shoots (biggest leaf on the shoot being an indicator of shoot size) (r^2 = 0.003, n.s., n = 381).

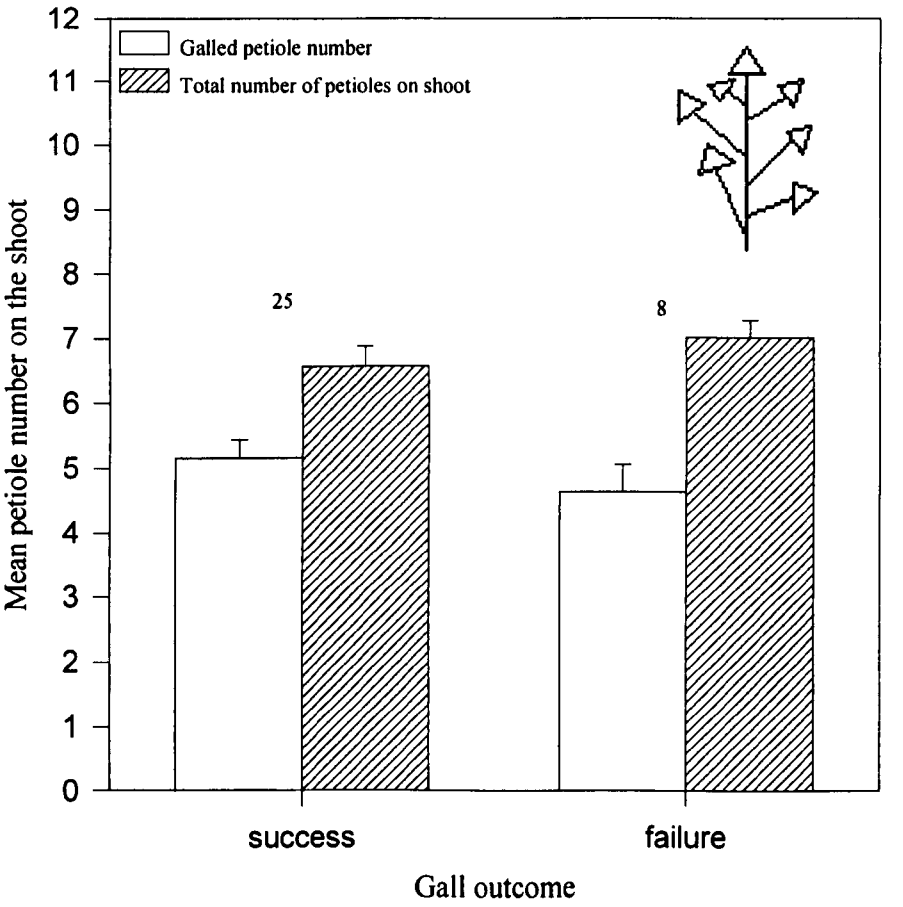
Figure 4. 3. 14



P. spyrothecae stem mothers did not congregate on petioles on leaves at any particular distance to the poplar tree trunk ($r^2 = 0.0004$, n.s., n = 764).

Figure 4. 3. 15

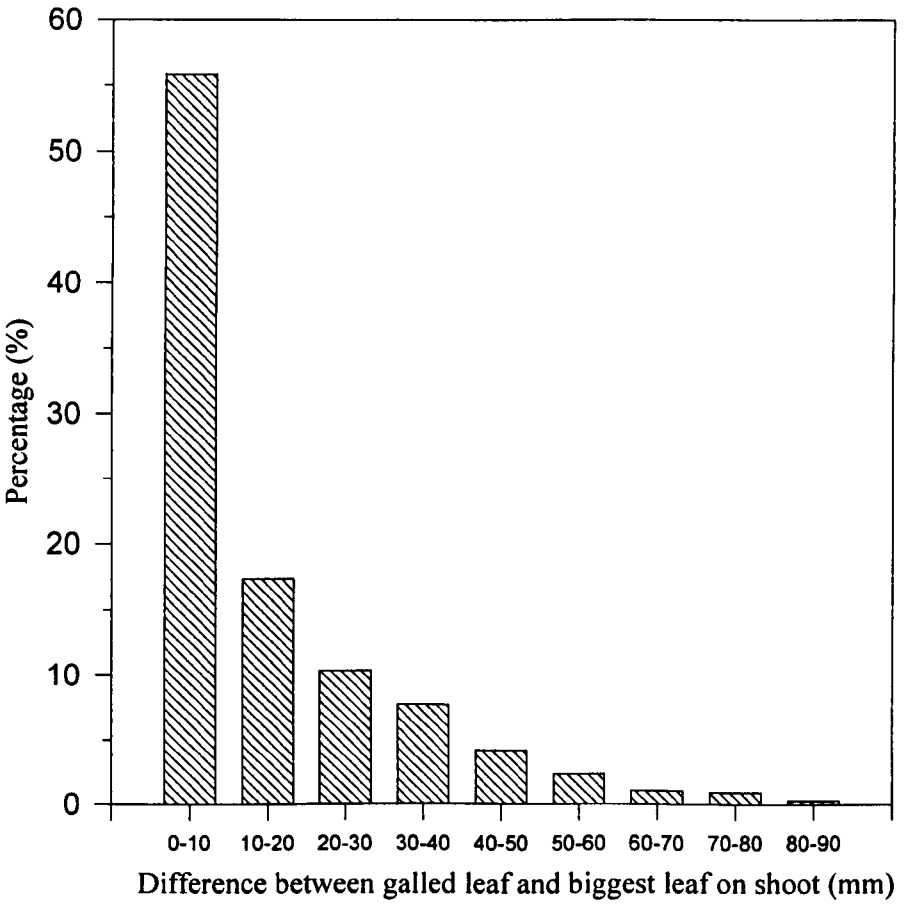
Successful and failed gall position on poplar shoots



Aborted *P. spyrothecae* galls appeared to be located, on average, further away from the top petiole on shoots with a greater average number of petioles per shoot. However, there was no significant difference between the position of the successful and the failed gall on the shoot (Mann-Whitney U = 71.0, n.s.) or between the size of these shoots (Mann-Whitney U = 93.0, n.s.). Petioles were numbered from basal to apical leaves (i.e. basal petiole = 1).

Figure 4. 3. 16

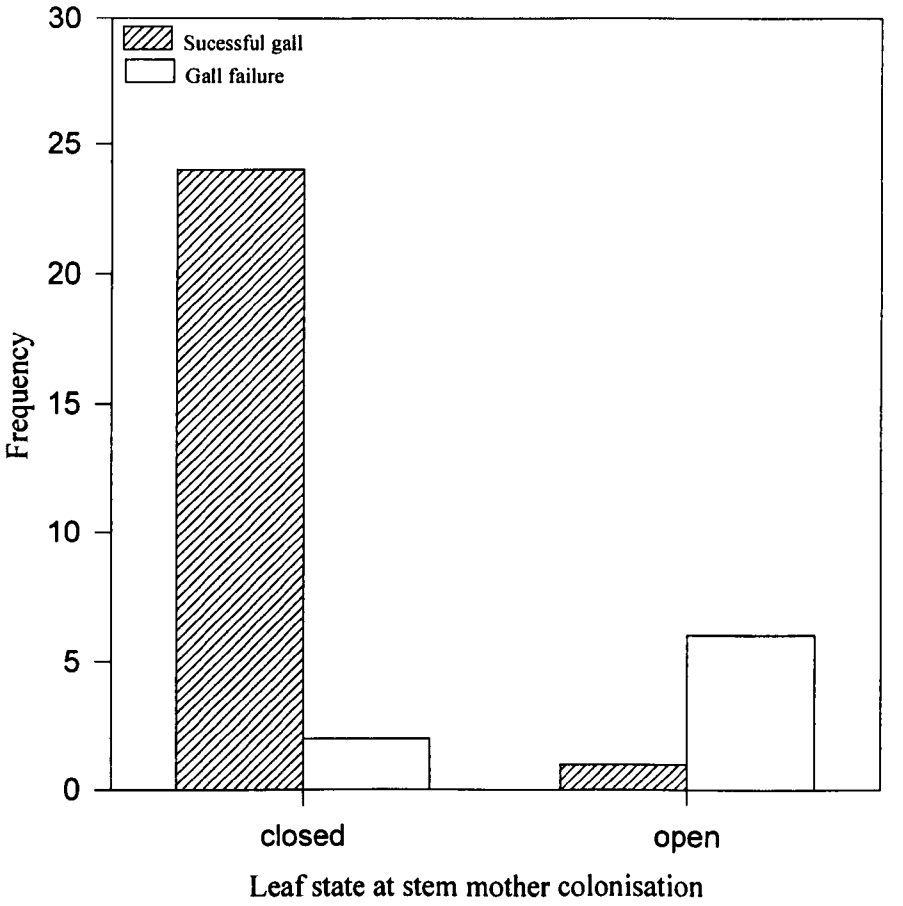
Difference in size between galled poplar leaf and biggest leaf on the shoot



At the time of colonisation *P. spyrothecae* stem mothers most often galled on a petiole where the leaf was closest in size to the biggest leaf on the shoot. 188 aphids out of 763 (24.6%) had galled on the biggest leaf.

Figure 4. 3. 17

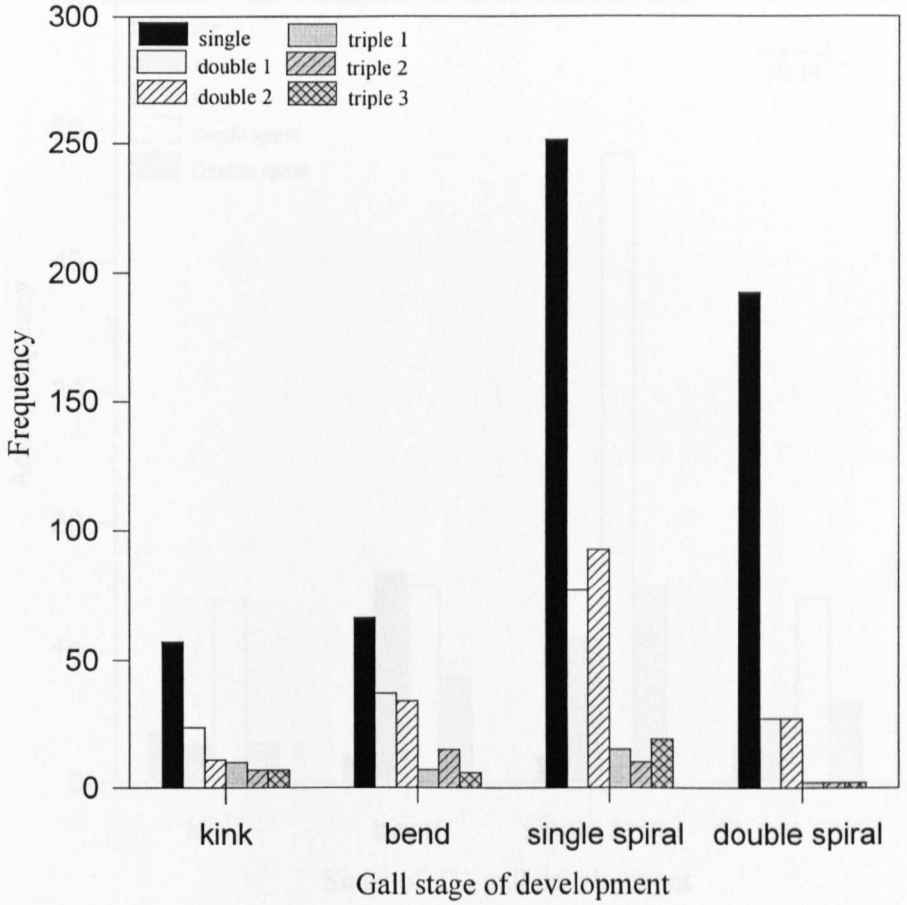
Aphids galling on petioles with open or closed leaves



P. spyrothecae stem mothers tended to gall on leaves that were not unfurled. Gall failure is more likey on petioles with open leaves ($\chi^2_1 = 15.9$, $p < 0.001$).

Figure 4. 3. 18

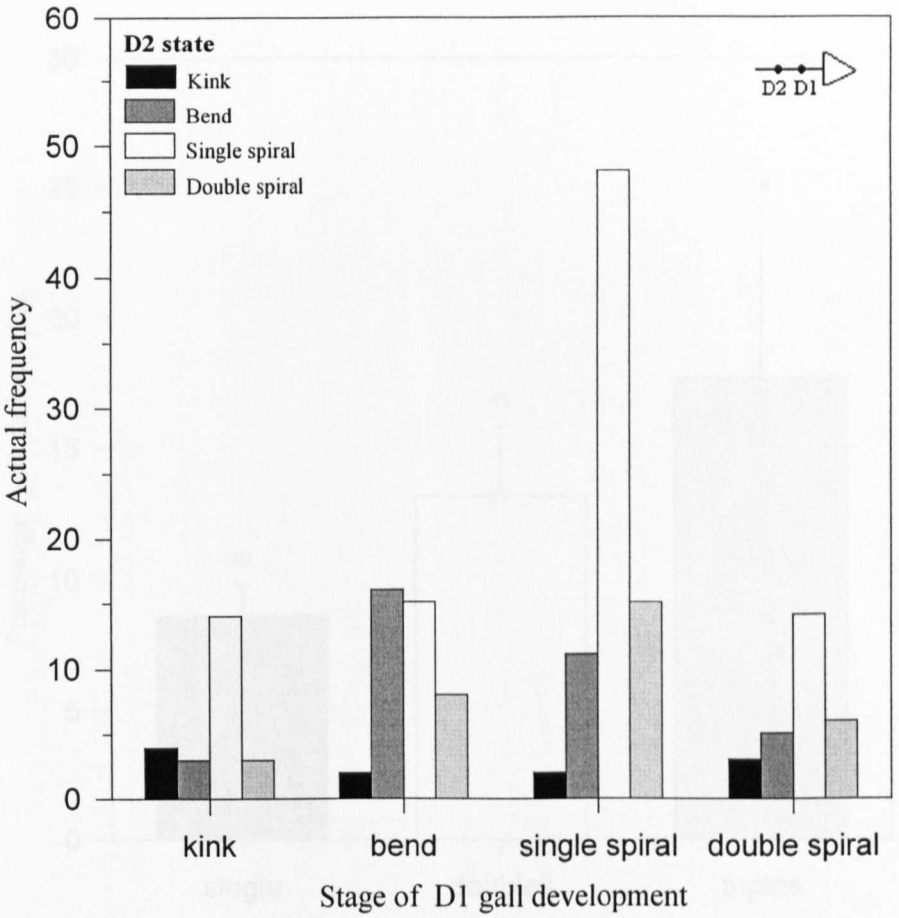
Gall stages of singly and multiply-galled poplar leaves



P. spyrothecae galls at different stages of gall formation on 15th May on singly or multiply-galled petioles. 34% of single galls had reached the completed stage compared to only 16.5% of doubles. A chi-square distribution revealed a significant association between galls per leaf and state of development ($\chi^2_{15} = 97.8$, $p << 0.001$).

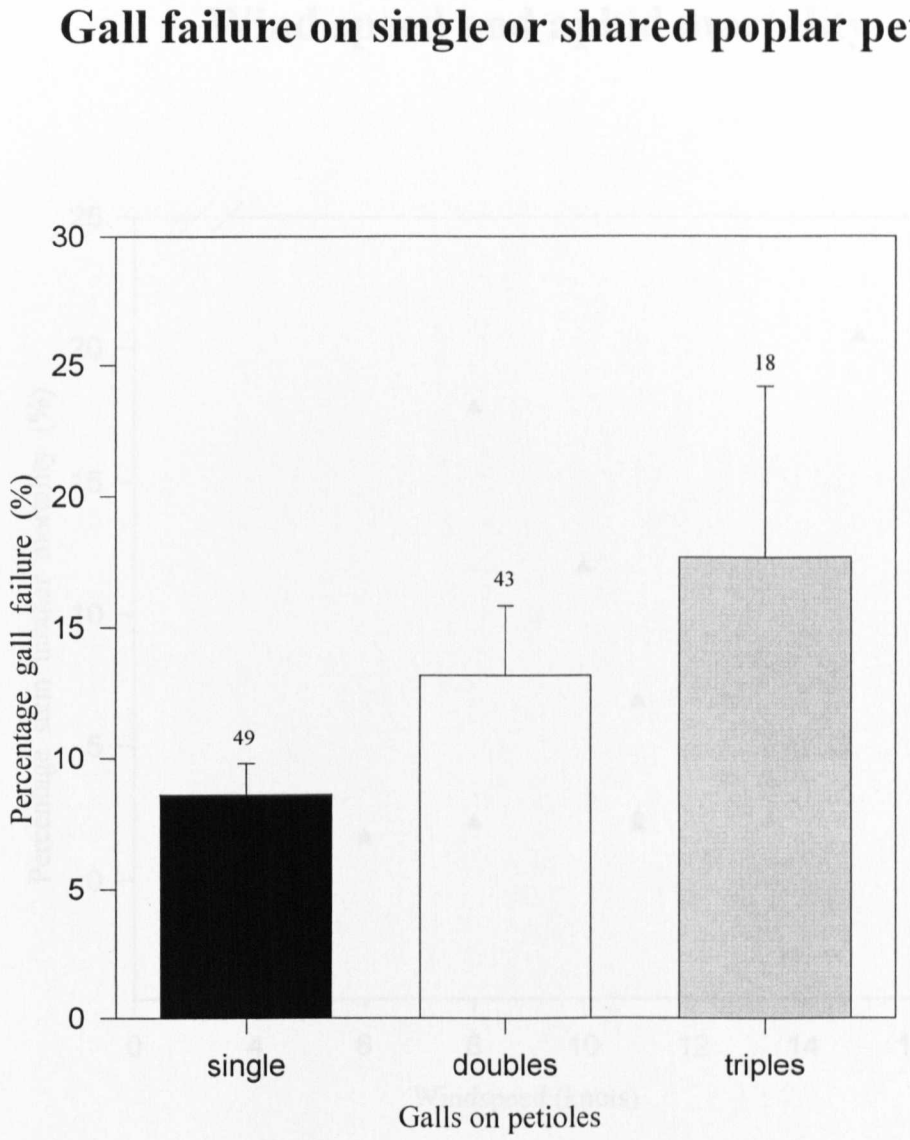
Figure 4. 3. 19

Stage of development of double *Pemphigus* galls



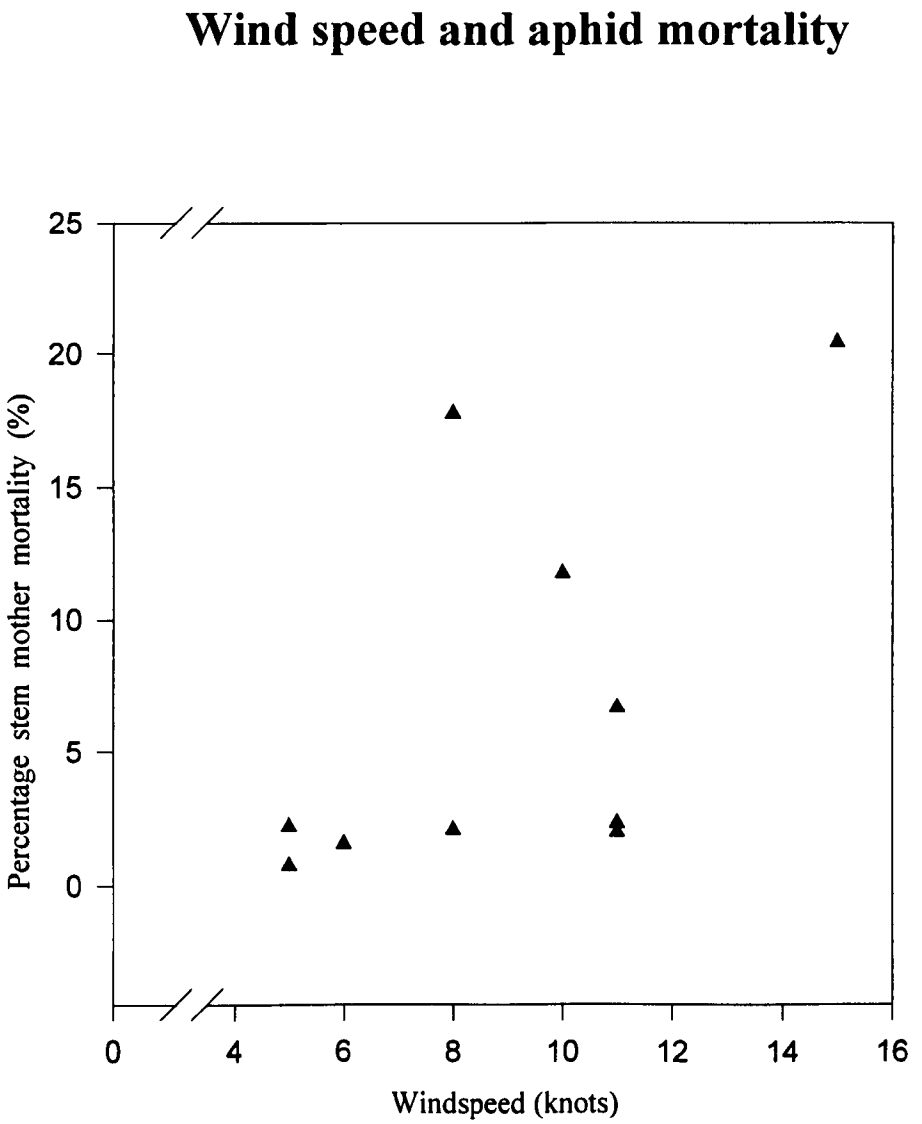
This illustrates the state of double galls at the time of census on 15th May, e.g. if the first of the double galls (closest to the leaf) was at the bend stage, 15 of the second double galls on that petiole had become single spirals. A chi-square distribution revealed a significant association between gall stages ($\chi^2_9 = 22.72$, $p < 0.01$).

Figure 4. 3. 20



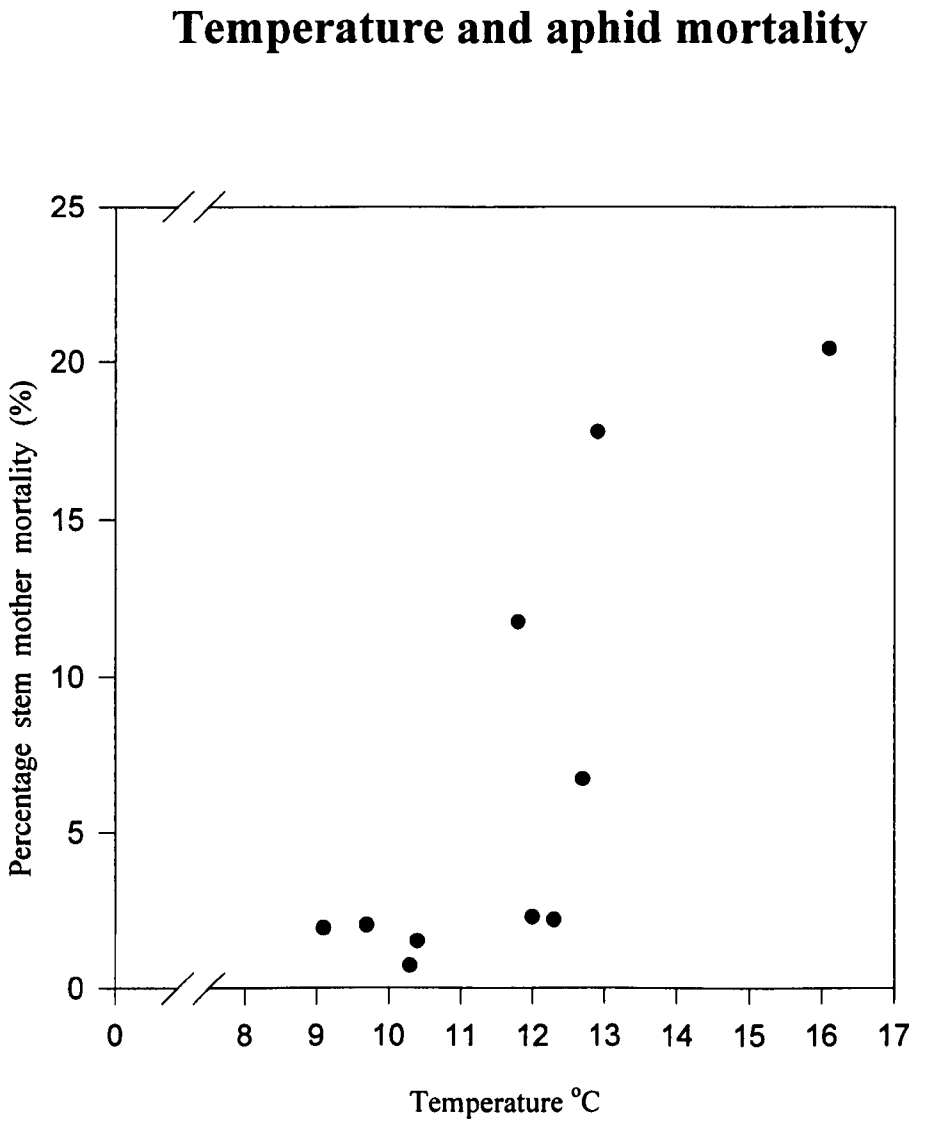
P. spyrothecae stem mother mortality on singly or multiply-galled poplar petioles at the time of gall initiation. Galls alone on the petiole were least likely to fail. Numbers refer to total number of gall failures.

Figure 4. 3. 21



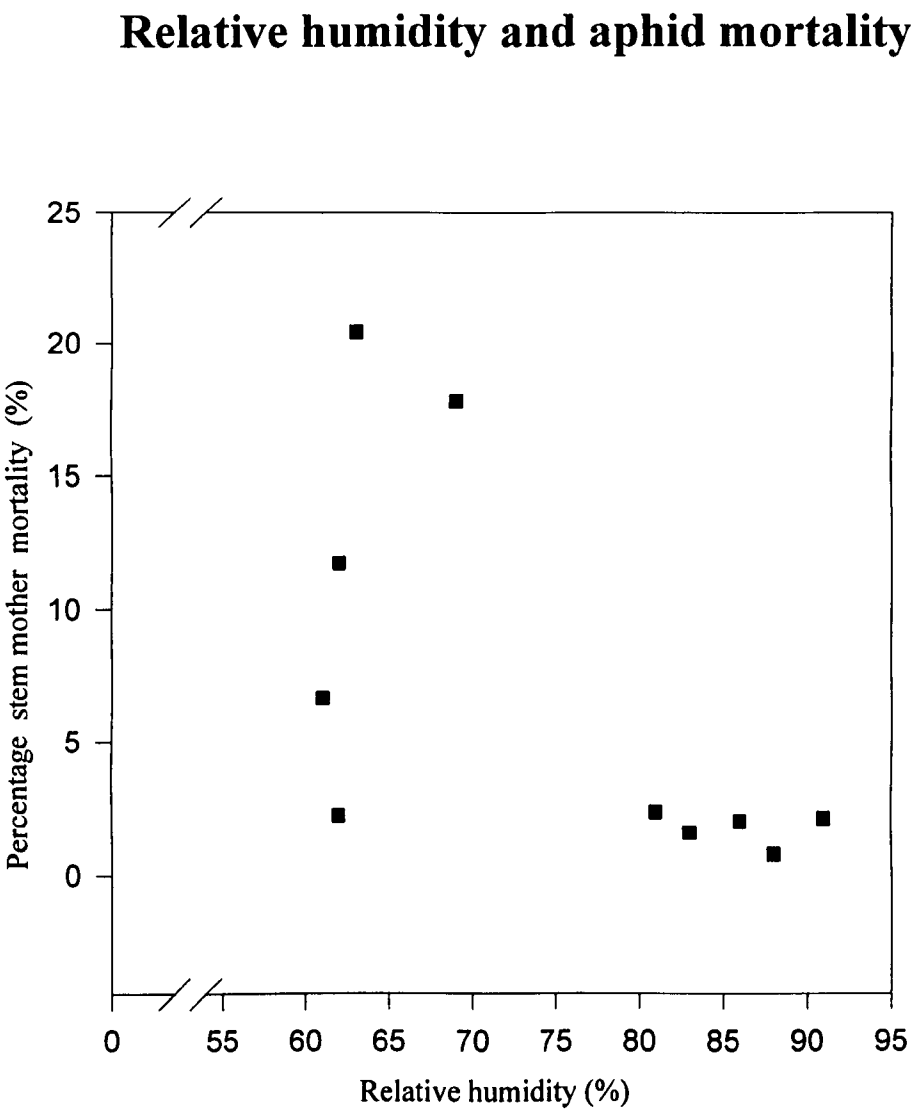
During April increasing wind speeds appeared to increase the percentage of dead *Pemphigus* stem mothers. This is, however, not statistically significant (partial correlation controlling for temperature, relative humidity, number of sun hours and amount of rain: $r^2 = 0.65$, n.s., n = 10).

Figure 4. 3. 22



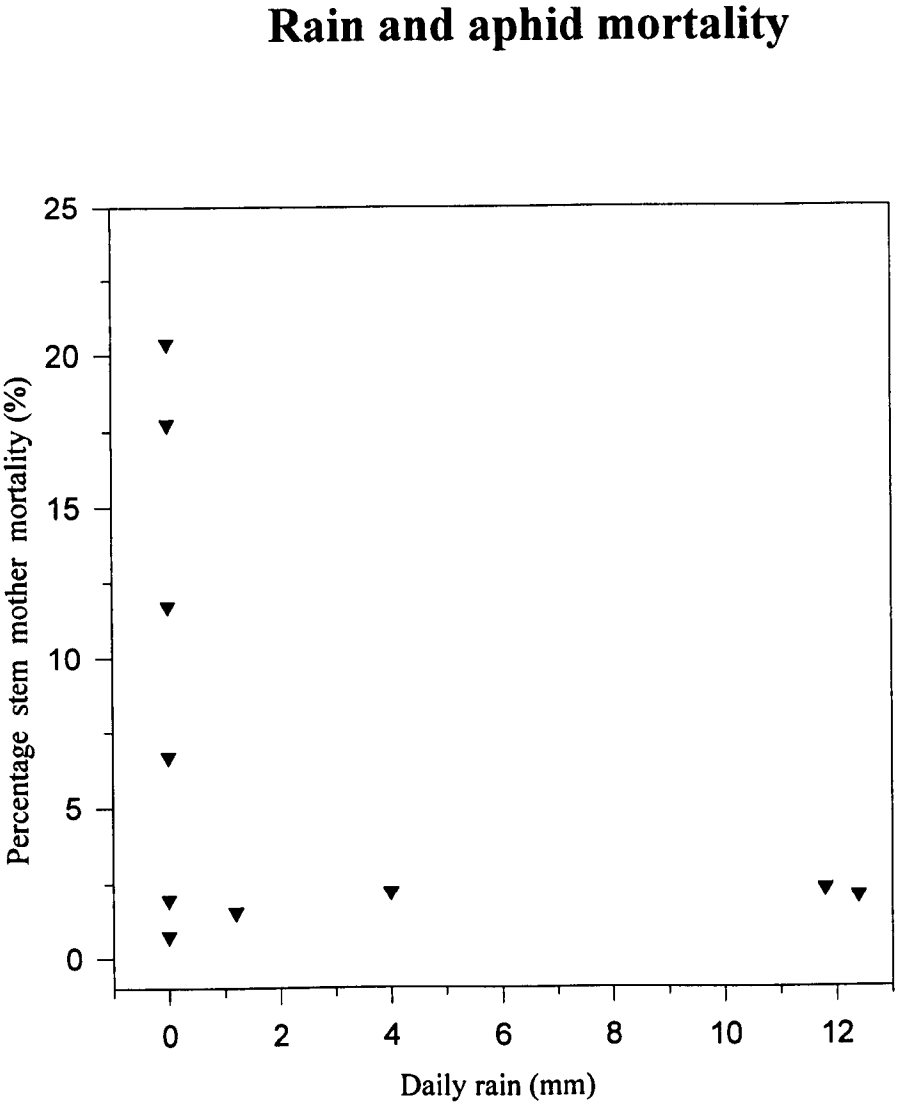
It appears that an increase in temperature increased *P. spyrothecae* stem mother mortality. However, controlling for other variables (number of sun hours, relative humidity, wind speed and rain) a partial correlation proved not significant ($r^2 = 0.42$, n.s., n = 10).

Figure 4. 3. 23



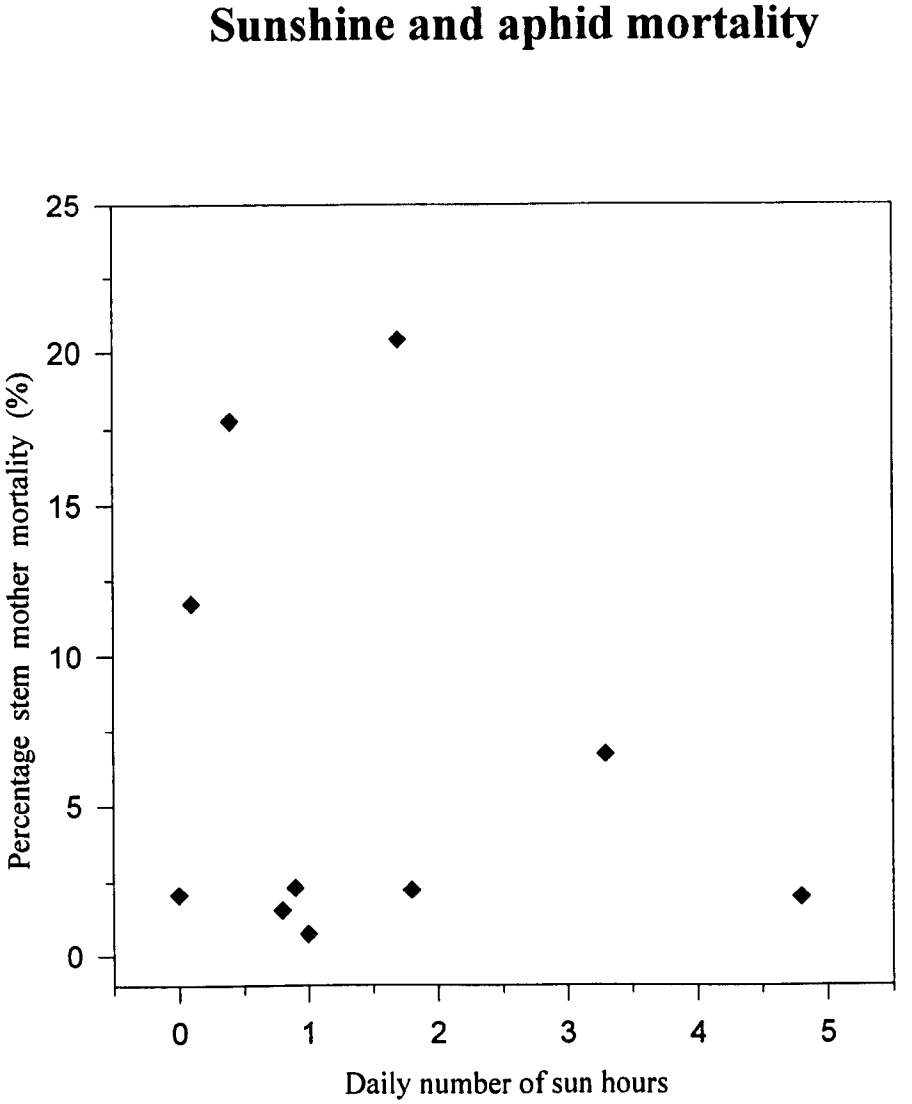
High relative humidity appeared to lower the percentage of dead *P. spyrothecae* fundatrices, but when controlling for temperature, wind speed, rain and sunshine, a partial correlation was not significant (r^2 = -0.045, n.s., n = 10).

Figure 4. 3. 24



Rain did not affect the survival of colonising *P. spyrothecae* stem mothers, a partial correlation controlling for sunshine, temperature, wind speed and relative humidity proved not significant (r^2 = -0.69, n.s., n = 10).

Figure 4. 3. 25



The number of sun hours during the month of April did not appear to affect *P. spyrothecae* stem mother mortality (partial correlation, controlling for relative humidity, rain fall, wind speed and temperature: $r^2 = -0.67$, n.s., n = 10).

Appendix 4. 3. 1a

Clone id.	D aphids placement 25 April	D aphids movement 28 April	D aphids settlement 12 May	D_2 aphids placement 28 April	D_2 aphids settlement 12 May
Dd1	3 on 3b[1] 3 on 5b	1⇧ on 10b 2 on base tb[2] 2 on tb 5/6[3] 1 on tb 6/6	1 abort tb 6/6		
Gd1	3 on 3b 3 on 5b	1⇧ on 7b 6/6	1 abort tb 6/7		
Hd1	3 on 3b 3 on 5b	2 on tb 6/7 1 on tb 7/7	1 gall tb 6/7		
Hd7				4 at 9cm 5 at 45cm	1 abort tb 5/7

1: 3b indicates bud three from top of clone
2: tb indicates top bud
3: 5/6 indicates leaf five out of a total six on the bud
⇧: indicates dead stem mother

Appendix 4. 3. 1b

Clone id.	G aphids placement 25 April	G aphids movement 28 April	G aphids settlement 12 May	G_2 aphids placement 28 April	G_2 aphids movement 29 April	G_2 aphids settlement
Dg1	3 on 3b 2 on 5b	3 on tb 6/6 2 galling	2 galls tb 6/6			
Dg2	2 on 3b	1 on tb 6/7	1 gall tb 6/7			
Gg1	4 on 3b 2 on 5b	1 on tb 5/7[5] 1 on tb 6/7[5] 1 on 2b 3/4[5] 2 on 4b 4/6[5] 1⇧ on 5b 2/5	1 gall tb 5/7 1 gall tb 6/7 1 abort 2b 3/4 1 gall 4b 6/6 1 gall 4b 5/6			
Gg2	2 on 3b	1⇧ on tb 5/7	no galls			
Gg3				7 at base 40cm		No galls
Hg1	2 on 3b 2 on 5b	1 on 4b 5/5 3 on tb 6/7	2 galls 4b 4/5			
Hg2	2 on 3b	2 on tb 5/6	2 galls tb 5/6			
Hg3				1 on tb 3 on 5b 3 on 10b	1 on tb 9/9 1 on tb 8/9 1 on 3b 5/5 1⇧ by 5b[4]	No galls
Hg4				6 at 3.5cm[6] 6 at 19cm[6] 6 at 35cm[6]		No galls

4: on transfer leaf near bud
5: forming gall
6: stem mothers at various states of gall formation (kink to single spiral) on transfer leaves

Appendix 4. 3. 1c

Clone id.	H aphids placement 25 April	H aphids movement 28 April	H aphids settlement 12 May	H_2 aphids placement 28 April	H_2 aphids movement 29 April	H_2 aphids settlement
Dh1	2 on 3b 1 on 5b	1⇧ on tb 2/6 1⇧ on tb 6/6 1 on 2b 4/4	No galls			
Dh2	1 on 7b	1⇧ on 4b	No galls			
Dh3	2 on 3b	1 on 5b 3/3	1 gall 5b 3/3			
Dh4				5 at 7cm[6] 10 at 24cm[6]		No galls
Gh1	2 on 3b 1 on 5b	1 on tb 6/7[5] 1 on 4b 5/5 1⇧ on 4b 5/5	1 gall tb 6/7 1 gall 4b 5/5			
Gh2	1 on 3b	1 on 3b 4/5	1 abort 3b 4/5			
Gh3				3 at 5cm[6] 5 at 33cm[6]	1 on 5b 2/5 1 betw 2-3b 1⇧ by 5b[4] 1⇧ by 10b[4]	No galls
Hh1	2 on 3b 1 on 5b	1⇧ on tb 4/5 1⇧ on tb 5/5 1 on tb 5/5	1 gall tb 4/5			
Hh2	1 on 3b	1 on tb 5/6	1 gall tb 5/6			

The first column (entitled **placement**) in each appendix indicates how many and where the *Pemphigus* fundatrices were placed on each clone, the second column (**movement**) shows how aphids had moved around the clone after 3 days, and the third column (**settlement**) records the final outcome. The subscript 2 (e.g. D_2) in the last columns refers to the second batch of translocation tests.

Appendix 4. 3. 2

Shoot	Leaf state[1]	Petiole[2]	Outcome	Date aphid seen (April)	Note
1.1	closed	5/7	gall	22	
1.4	closed	7/7	gall	25	
1.5	closed	6/6	gall	26	
1.5	open	4/6	gall failure	24	
1.7	closed	6/7	gall	28	
2.3	closed	6/8	gall	26	
2.3	open	5/7	gall failure	24	
3.6	closed	6/6	dead	25	
3.10	closed	2/3	gall	25	
5.1	closed	6/7	gall	28	
6.1	open	4/8	gall failure	24	
6.4	closed	6/6	gall	28	
6.7	closed	4/5	gall	25	
6.10	closed	6/7	gall	25	
7.5	open	4/7	gall	24	
7.7	closed	6/6	gall	26	
7.10	closed	7/8	dead	27	
8.6	closed		dead	24	bud not open
9.1	closed	3/8	gall	25	
9.1	closed	4/8	gall	27	
9.1	closed	5/8	gall	27	
10.5	closed	3/3	gall	28	
10.6	closed	7/7	?	27	walking
11.4	open	3/7	gall failure	25	
11.4	open	4/7	gall failure	27	
11.4	closed	5/7	gall	26	
11.5	open	5/7	gall failure	26	
11.6	closed	7/7	?	26	walking
11.8	closed	2/3	?	27	walking
11.9	open	2/3	?	26	walking
12.4	closed	5/8	gall	24	
15.1	closed	7/8	gall	26	
16.1	closed	6/7	gall	26	
18.2	closed	6/7	gall	22	
18.3	closed	7/8	gall failure	27	
18.4	closed	6/6	gall	26	
18.9	closed	5/6	gall failure	26	
18.10	closed	5/6	?	25	walking
19.2	closed	5/7	gall	24	
20.5	closed	4/8	dead	24	
20.5	closed	7/8	gall	27	
20.6	closed	3/3	gall	26	

1: *leaf state* refers to whether the leaf is unfurled or not
2: *petiole* refers to the location of the aphid stem mother. e.g. 4/8 means on the 4th of 8 petioles on the shoot

Chapter 5

THE PHENOLOGICAL BACKGROUND TO APHID NUMBERS IN THE GALL

5. 1 Introduction

Density-independent environmental factors (Dempster, 1983; den Boer & Reddingius, 1989) and stabilizing density-dependent factors (Gradwell & Hassell, 1973; Latto & Bernstein, 1990; Turchin, 1990; Dixon *et al*, 1986; Jarośik & Dixon, 1999; Guldemond, van den Brink & den Belder, 1998) affect aphid populations causing them not to reach their maximum potential growth. Reaching maturity within 14 days, aphids are able to multiply rapidly (approximately 30 nymphs are born to each aphid) and soon reach pest proportions (Dixon, 1985). During favourable environmental conditions some aphids are capable of rearing nine generations, which means one aphid could produce over $600x10^9$ aphids, and as each aphid weighs roughly 1 mg, this amounts to 600 000 kg of aphids in one season, the equivalent of 10 000 men. This growth rate is only possible under optimum weather conditions with unlimited resources, and the absence of parasites, pathogens and predators. However, under natural conditions the above regulatory factors inhibit population growth (Dixon, 1985).

Changes in aphid population density (Wool & BenZvi, 1998; Dixon, 1985; Dixon *et al*, 1996), in host plant quality (Whitham, 1978; Agarwala & Datta, 1999; Guldemond, van den Brink & den Belder, 1998) or the onset of adverse environmental conditions can often trigger alate production, whose primary role is to colonise new plants. Not only does predation by insects such as ladybirds, hoverfly larvae and flower bugs strongly influence aphid population growth rates (van den Berg *et al*, 1997), but the presence of a predator may directly increase the number of winged morphs in a population (Weisser, Braendle & Minoretti, 1999). Moving between habitats is costly, because many migrants never reach a new host plant and/or overwintering site. However, the suitability of a new environment for population growth should exceed that of the current habitat (Southwood, 1977).

In galling aphids large females may through competitive interaction usurp other stem mothers attempting to colonise the same favourable leaf, forcing smaller sized stem mothers to share less suitable leaves and thus reduce their fecundity relative to single stem mothers (Whitham, 1986). On leaves with two fundatrices, basal stem mothers have been found to be over six percent larger than their distal competitor (Whitham, 1979) and produce over 50% more progeny, which develop faster (Whitham, 1978) than fundatrices displaced to inferior distal sites (Whitham, 1986).

Host plants are known to differ in their resistance to insect herbivore attack (Watt, 1990; Moran & Whitham, 1990; Memmott *et al*, 1995; Edmunds & Alstad, 1978; Rohfritsch, 1981; Stoetzel & Tedders, 1981; Wool, 1984). The quality of the host plant is vital because it determines the rate of increase or decline of aphid populations (Bommarco & Ekbom, 1996; Jarośík & Dixon, 1999; deKogel *et al*, 1997). By host shifting some herbivores have obtained a higher population growth rate and a shorter immature developmental period (Shirai & Morimoto, 1997).

Using data on the growth of galls and of aphid numbers within the gall during the season, several questions can be addressed: a) what are the average growth and mortality rates of *Pemphigus spyrothecae* galls and the enclosed wingless and alate aphids (also refered to as juveniles and adults in this chapter); b) how does predation influence aphid mortality?; c) do large stem mothers produce more offspring at a faster rate than small stem mothers; d) if stem mothers share a petiole with another fundatrix, how does this affect the aphid numbers in both galls; and e) since host plants are known to vary in their susceptibility to herbivore attack, does the host tree influence the rate at which the aphid colony grows?

5. 2 Methods

The life cycle of *Pemphigus spyrothecae* Passerini, 1860 has been described by Dunn (1966) and Foster (1990). This species is not host alternating, but spends its whole life cycle on its primary host, the black poplar, *Populus nigra*, and its hybrids and varieties. Eggs laid the previous year in the bark on branches or on the trunk of the host tree, hatch in spring and produce parthenogenetic stem mothers (first generation). The wingless stem mothers are very mobile, and migrate out to the newly emerged shoots, where they select a petiole for gall formation. By midsummer up to several hundred offspring are parthenogenetically produced. The first instars grow into wingless adults (virginoparae, second generation), and these in turn produce both thick-legged and normal-legged first instars. The normal-legged instars grow into winged sexuparae (third generation), that, once the galls have opened in August, migrate to the trunk, give birth to sexuals (fourth generation), which mate and produce overwintering eggs in the bark of the tree.

The ten black poplars, *Populus nigra* var. *italica*, used in this study are located on the campus of Nottingham University. 10 singly and 10 doubly galled leaves were collected unbiasedly from each tree once a week for five weeks starting 9th July, encompassing most of the growth period of the gall up to the point just before it opens and the alates disperse. 50 singly and 25 doubly galled leaves were collected from each of the 10 trees in the final sixth week when galls were just beginning to open. For ease of collection, galls were taken from the lowest three metres of the trees.

Each week gall wet weight was recorded in mg on a Mettler PJ 400 weighing machine. Counting the number of progeny per gall indicated the individual fitness of each stem mother. The galls were opened and the occupants gently transferred with a small paintbrush into a petri-dish, and the number of aphids (wingless and alates) counted under a Wild M3 dissecting microscope. If the stem mother was identifiable, her length was measured in mm. The number of dead aphids and any non-aphid inhabitants or predators were noted and retained for identification.

Where appropriate, in order to eliminate the effects of among tree variation, variables were standardised. Sample sizes and standard errors are included where possible in graphs using mean values. Variables were tested for normality, and where necessary, non-parametric statistics were used. To avoid the effects of competition, only leaves containing a single gall were used in some analyses. The effects of gall order on fitness components were analysed as a paired design by using the differences between the distal gall values and the proximal gall values as the data set.

5. 3 Results

Single *Pemphigus spyrothecae* gall weights exhibited a growth rate of 80% during the six week period starting 9th July; their weight averaged just over 250 mg in week one and 450 mg in week six (Fig. 5.3.1). Within single galls the number of offspring grew steadily during the study period, ranging from an average of 14 in the first week to nearly 100 wingless aphids per gall in the sixth week, and this change was statistically significant (Fig. 5.3.2). Alates were not produced until the third week (week commencing 30th July), and by the sixth week a rapid production resulted in nearly 10 alates per gall (Fig. 5.3.3). Overall there was a significant rise in aphid density in galls during the study period (Fig. 5.3.4), and as the number of wingless adults increased, more alates were produced (Fig. 5.3.5). There was a weak positive relationship between galled leaf area and number of alates produced per gall ($r^2 = 0.032$, $p < 0.0001$, $n = 500$).

It is not uncommon for a gall colony to be wiped out, either due to adverse weather conditions or predation. Gall demise on the poplars in this study did not appear to vary much among weeks, with the highest percentage being in the first week (6%) and the lowest in the second week (2%) (Fig. 5.3.6). As the weeks passed, although subject to fluctuation, the average percentage mortality of aphids in galls increased (Fig. 5.3.6). The mortality rate ranged from 5.3 to 18.1% during the six week period. This general increase in mortality may be due to the sharp increase in predators towards the last few weeks of the study (Fig. 5.3.7). Aphids experienced the highest mortality rate in week five which coincides with the week having the highest mean number of *Anthocoris minki* per gall: there was a significant relationship between percentage aphid mortality and predation ($r^2 = 0.381$, $p < 0.0001$, $n = 1000$). Predators were found within galls in 53.5% of gall demises.

Larger *P. spyrothecae* stem mothers produced heavier galls in the first week (9th July) (Fig. 5.3.8), but this relationship did not persist in the final week. No relationship was detected between stem mother size and number of offspring per gall (Fig. 5.3.9). However, in both the first and final weeks of study, the probability values approached significant ($p < 0.06$ and $p < 0.07$, respectively); a larger sample size may have

confirmed this relationship. In week four the number of aphids per gall doubled as stem mother size increased from 1.75-2 mm to 2-2.25 mm.

Aphid mortality rates fluctuated during the study period; in some stem mother size intervals (e.g. 0-1.75 mm) the average percentage mortality rose steadily as the weeks passed, whilst in other categories average mortality rose then fell towards the end of the study period: there was no relationship between stem mother size and aphid mortality (Fig. 5.3.10). Interestingly, the average size of *Pemphigus* stem mothers generally decreased during the six week study period, and this was the case for both singly and doubly galled stem mothers (Fig. 5.3.11). There appeared to be a weak but significant relationship between stem mother size and the size of the occupied leaf (Fig. 5.3.12).

P. spyrothecae stem mothers sharing a petiole did not differ in size (Fig. 5.3.13). With the exception of the fifth and sixth weeks (6th and 13th August), proximal galls (closest to the leaf junction) tended to be heavier than distal galls (furthest from the leaf). There were significant effects of gall order on gall growth (Fig. 5.3.14). Although the average distal gall was lighter than the proximal gall in the first week, the distal gall had a 24% higher growth rate than the proximal over the six week period. As expected the number of wingless aphids in each gall increased as the weeks passed, but gall order had no effect on the increase in the number of juvenile aphids per gall (Fig. 5.3.15). The production of alates in the double galls did not begin until the fourth week of the study (30th July onwards) (Fig. 5.3.16), and there appeared to be no effects of gall order on the growth in alate numbers. Overall, there were no significant effects of gall order on the growth in number of aphids in the gall (Fig. 5.3.17). The average mortality of *P. spyrothecae* aphids in double galls ranged between 15-25%, and there appeared to be little difference between proximal (closest to the leaf) and distal galls in any week (Fig. 5.3.18).

The average gall generally got heavier on all ten *Populus* trees as the weeks passed. There were significant effects of trees and weeks on gall weights and a difference in the rate of gall growth among trees during the study period (Fig. 5.3.19). Likewise, the average number of wingless aphids per gall differed among trees and weeks (Fig.

5.3.20). However, although the number of alates differed among trees and weeks, no interaction between trees and weeks on the adult aphid density per gall was evident (Fig. 5.3.21). Overall, there was a significant difference in the growth rates of aphid density per gall among trees (Fig. 5.3.22), but this was not reflected in aphid mortality rates among trees in any week (Fig. 5.3.23).

5. 4 Discussion

Aphid population densities increase and decrease throughout a season (Jarośík & Dixon, 1999; Dixon *et al*, 1986; Guldemond, van den Brink & den Belder, 1998) and are subjected to density-dependent and independent factors such as environmental and predation pressures (van den Berg *et al*, 1997). When the population numbers are high, aphids are able to regulate their own density by dispersal and by reducing their fecundity (Dixon, 1985). Under field conditions aphid numbers usually decline rapidly after reaching high numbers, and although the population may increase later in the season, it is limited by the low temperature and short day conditions which induce the development of sexual forms and the laying of overwintering eggs.

Many insects show a spring and autumn population growth pattern which can in part be explained by host plant development (van den Berg *et al*, 1997; Guldemond, van den Brink & den Belder, 1998). In spring when host plants are actively growing and in autumn when leaves are approaching senescence, the phloem sap contains relatively high concentrations of nitrogen (Dixon, 1985). If nitrogen and phosphorous levels in the food resources are reduced, fecundity and population growth also reduce regardless of the quantity of food available (Kilham, Kreeger, Goulden & Lynn, 1997). Examples of the regulatory effect of host plants include the monophagous sycamore aphid, where both the size of the aphid and its reproductive performance are determined by the quality of the host plant (Dixon, 1985) and the mustard aphid *Lipaphis erysimi*, which regulates its developmental period, fecundity and population growth in response to developmental changes in the mustard plant (Agarwala & Datta, 1999). The gall tissue expanded greatly to accommodate the almost 7-fold increase in the numbers of offspring of *Pemphigus spyrothecae* aphids during the six week study period. Wool & BenZvi (1998) noted that as the *Geoica wertheimae* clone size increased (from one individual to several hundred aphids per gall) during the season, the gall tissue increased eight fold in volume.

During the fifth week the number of *P. spyrothecae* aphids appeared to have reached a maximum, coincident with the start of maturation of alates as the time for dispersal approached. The stabilising factor influencing population growth in other aphid

systems is likely to be the cessation of plant growth; the concentration of amino-acids in the phloem sap declines, the nymphs develop into progressively smaller adults, the birth rate drops, and when the leaves are mature, reproduction may cease altogether (Dixon, 1985).

In many aphid systems, an abrupt change in aphid density within the galls appears to trigger the induction of the alate morph in galls in late summer. This usually occurs when the aphid reproductive rate exceeds the rate of change in gall internal surface area (Wool & BenZvi, 1998). Other factors have also been known to induce the development of alate forms: quality of host plant, crowding, temperature and photoperiod and ant attendance (Dixon, 1985). As in the present study, Whitham (1978) showed that the galled leaf size affected the rate at which the offspring develop to become adult winged migrants.

During the study period, the frequency at which the *P. spyrothecae* gall colonies were wiped out varied between an average of 2 and 6% in the sampled population, and although a rise in the average aphid mortality rate was seen in the fifth week, this can be explained by the abrupt increase in predator numbers. Predators are usually not common in the first few weeks following budburst, but by the time adult migrants appear and are dispersing, a third of all galls containing aphids may be preyed upon by one or more predators (Whitham, 1978). Aphids are attacked by a wide range of predators and predation has been shown to influence the growth and persistence of aphid colonies. For instance, coccinellid predators play a major role in suppressing *Aphis glycines* in soybean (van den Berg *et al*, 1997), and whereas herbivore populations with a slower growth rate may be exposed to parasitism over a longer period and so experience a higher rate of parasitism (Clancy & Price, 1987), a predator-induced defense such as the production of alates does not necessarily lead to better protection against predator attack, but enables aphids to leave plants when mortality risks are high (Weisser, Braendle & Minoretti, 1999).

Predators such as ladybirds and hoverfly larvae are not the only insects which affect the population dynamics of aphids. The presence of Collembola reduced aphid reproduction on *Trifolium repens* by 45%, but on another host plant, *Poa annua*,

increased offspring production by a factor of 3 (Scheu, Theenhaus & Jones, 1999). It appears that springtails decrease aphid reproduction on more palatable host plants like *T. repens*, but by slightly increasing the nitrogen concentration in plant tissue, make less palatable ones such as *P. annua* more favourable for aphid reproduction. Even the presence of earthworms in the soil has been found to increase aphid population densities by up to 70% (Scheu, Theenhaus & Jones, 1999).

Galling aphid fundatrices vary in size and this variation affects reproductive fitness (Fay & Whitham, 1990; Whitham, 1978). Although initially there was a relationship between the size of *Pemphigus spyrothecae* stem mothers and gall weight, but by the 13th of August this relationship had ceased. Within the galls, the offspring of small stem mothers also suffered a constant increase in percentage mortality as the weeks passed. Although the mortality rate of *Adelges cooleyi* fundatrices in the top-most spruce whorls was significantly higher than on other whorls (the rising sap in the early developing whorl makes aphids more susceptible to freezing), the average body weight of the stem mothers was over seven times greater in whorl 1 than in whorls 8-10 (Fay & Whitham, 1990). Not only was the growth rate of stem mother survivors the highest on spruce whorls (whorl 1) which generated the longest shoots, but *Adelges cooleyi* stem mothers also gave birth to the greatest number of progeny and the heaviest offspring (Fay & Whitham, 1990). Similarly to Whitham's (1978) results, there was a significant relationship between *P. spyrothecae* stem mother size and poplar leaf area.

Whitham (1986) suggested that differences in body sizes of first instar colonising stem mothers are more likely to be related to dominance or competitive ability than to reproduction. Through territorial competition larger *P. betae* stem mothers (an average difference of only 3.4% in prothorax widths) usually win the favoured site at the base of the leaf and here produce an average of 58% more progeny than smaller stem mothers displaced to inferior distal sites (Whitham, 1986). In this study the order of *P. spyrothecae* galls determined aphid colony growth rates: the distal gall (furthest from the leaf) had a higher growth in aphid numbers during the six week period examined. Although Whitham (1978) recorded a higher developmental rate of aphids at the base of the leaf where phenolics are at their lowest concentrations (Zucker, 1982), I found

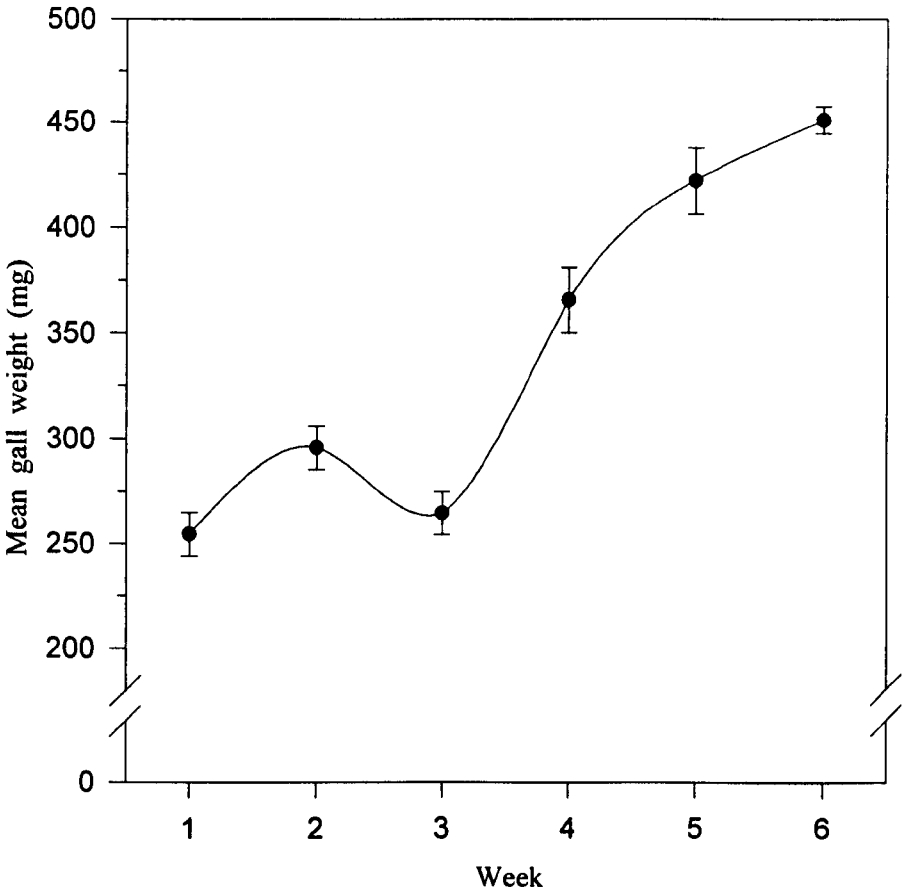
no difference between double galls in the rate at which alates were produced. Interestingly, if stem mothers are released from the negative effects of competition, differences in body size have no effect on the subsequent production of progeny (Whitham, 1986).

Host plant habitat was the most important variable determining the rates of increase and decline of pea aphid populations (Bommarco & Ekbom, 1996) and *Frankliniella occidentalis* (thrips) populations (de Kogel *et al*, 1997). Jarośik & Dixon (1999) recorded density-dependencies throughout the season on one tree, whilst on another tree, this was notable only towards the end of the season when aphid densities were low. The different seasonal abundances on the two trees may be a consequence of differences in phenology of the trees. Although I found a significant effect among poplar trees in the growth rate of both gall and the number of wingless aphids per gall, the production rate of alates did not vary among trees, indicating that abiotic or perhaps intrinsic factors most likely trigger the induction of alates.

In conclusion, the gall tissue grew quickly to encompass the rapid increase in the number of aphids. There was little difference between mortality rates of aphids in distal and proximal galls (closest to the leaf), but aphid mortality was related to predation. During the six week study period, stem mother size and gall order on doubly-galled petioles had little effect in the number of progeny produced per gall. However, host plant played a significant role in gall growth and rate of aphid production.

Figure 5. 3. 1

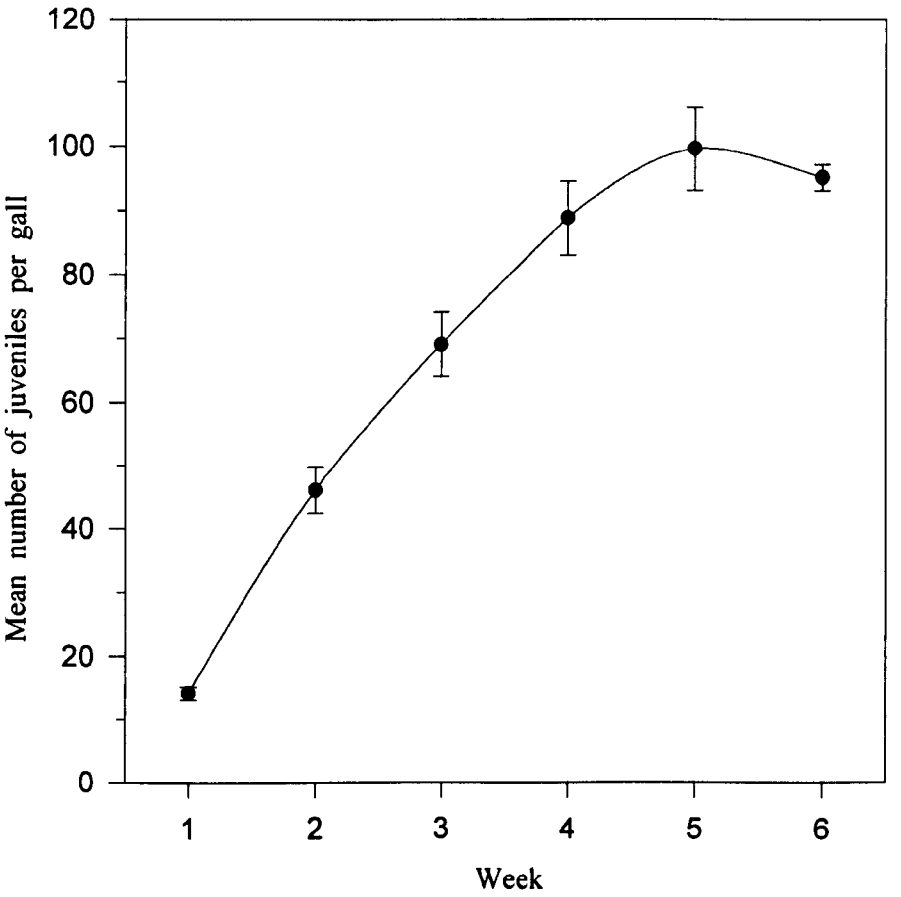
P. spyrothecae gall weight growth



Change in mean gall weights during a six week period starting 9th July. There was a significant effect of weeks on single gall weights (ANOVA $F_{5,999} = 66.84$, $p < 0.0001$). Weeks 1-5: n = 100; week 6: n = 500.

Figure 5. 3. 2

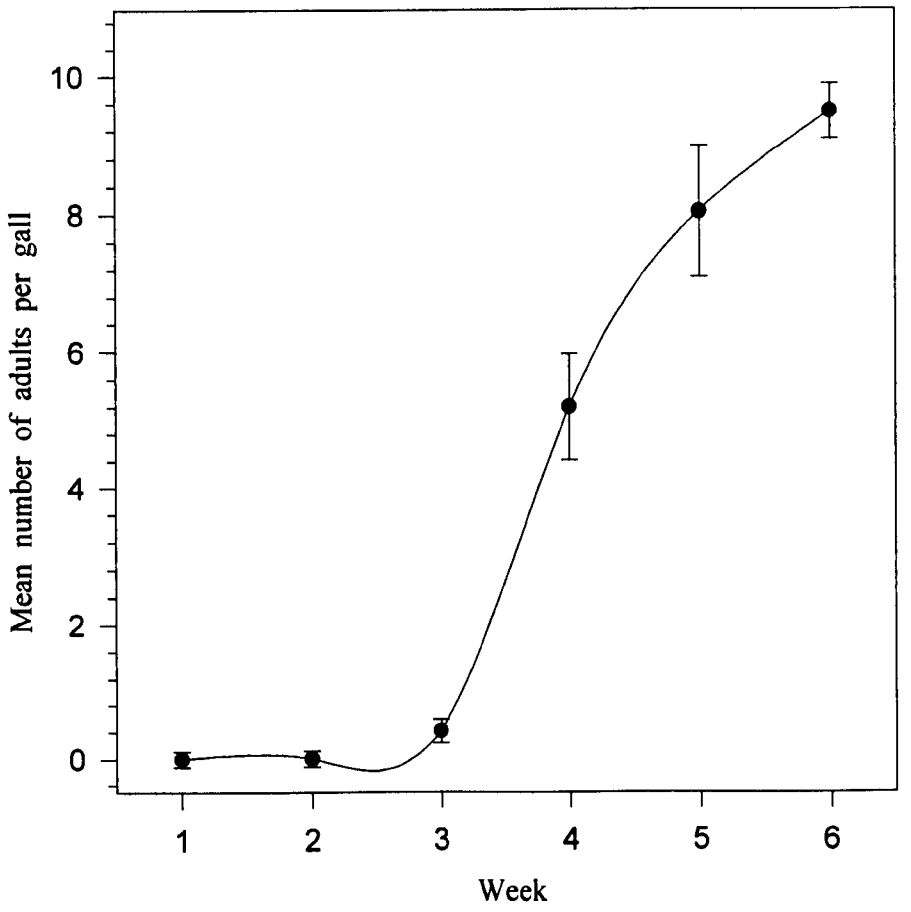
Change in the number of *P. spyrothecae* wingless aphids per gall



Change in the average juvenile density per single gall during a six week period commencing 9th July. There was a significant change in density (ANOVA $F_{5,999}$ = 66.86, $p < 0.0001$). Weeks 1-5: n = 100; week 6: n = 500.

Figure 5. 3. 3

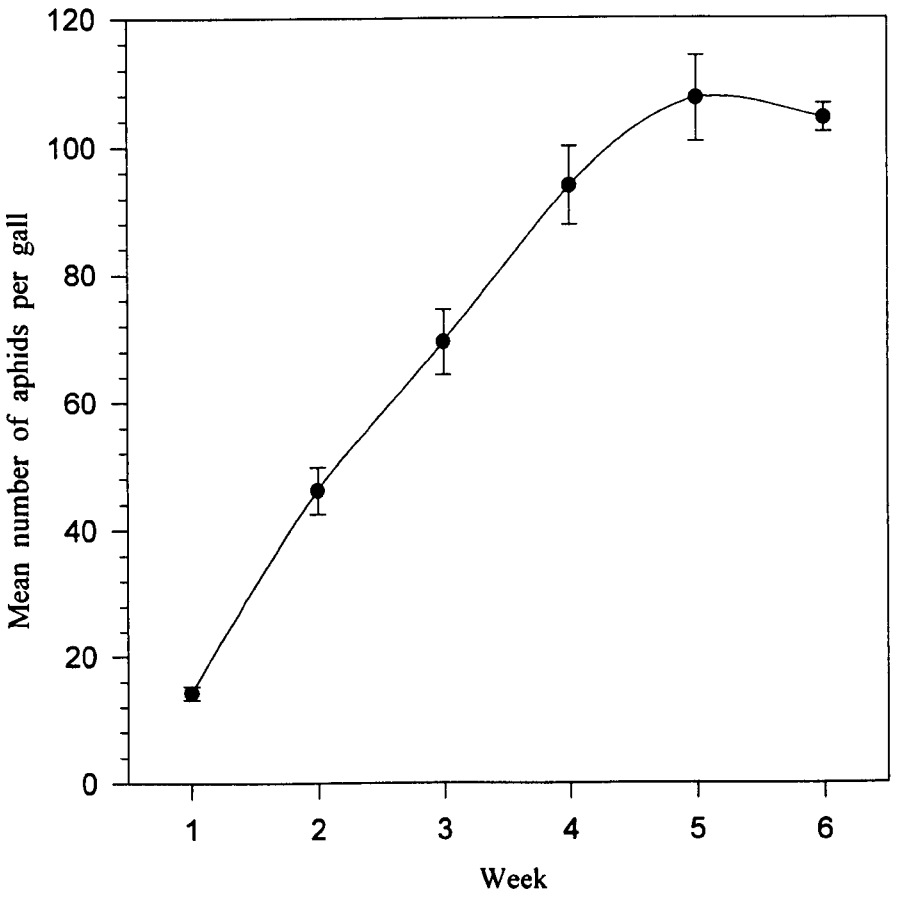
Change in the number of *P. spyrothecae* alates per gall



Change in the adult density per single gall during a period of six weeks commencing 9th July. There was a significant effect of weeks on adult density (ANOVA $F_{5,999}$ = 59.44, $p < 0.0001$). Weeks 1-5: n = 100; week 6: n = 500.

Figure 5. 3. 4

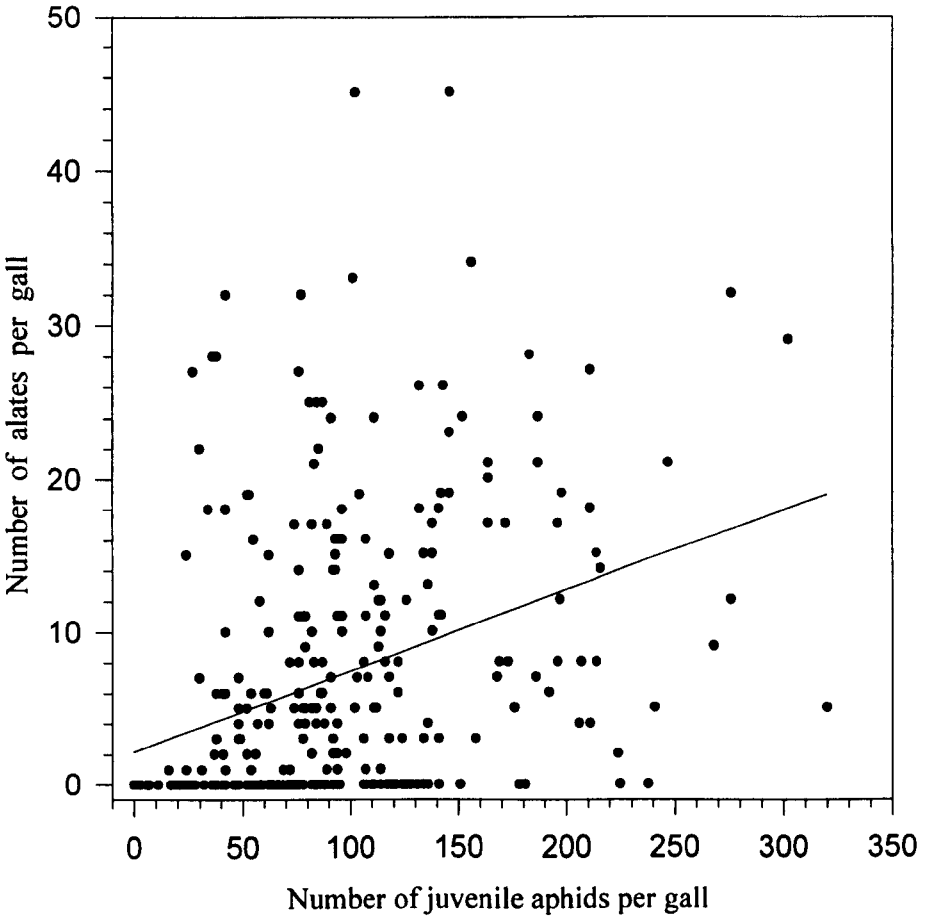
Change in the number of *P. spyrothecae* aphids per gall



Change in the aphid density per single gall during a period of six weeks commencing 9th July. There was a significant change in aphid density (ANOVA $F_{5,999} = 76.01$, $p < 0.0001$). Weeks 1-5: n = 100; week 6: n = 500.

Figure 5. 3. 5

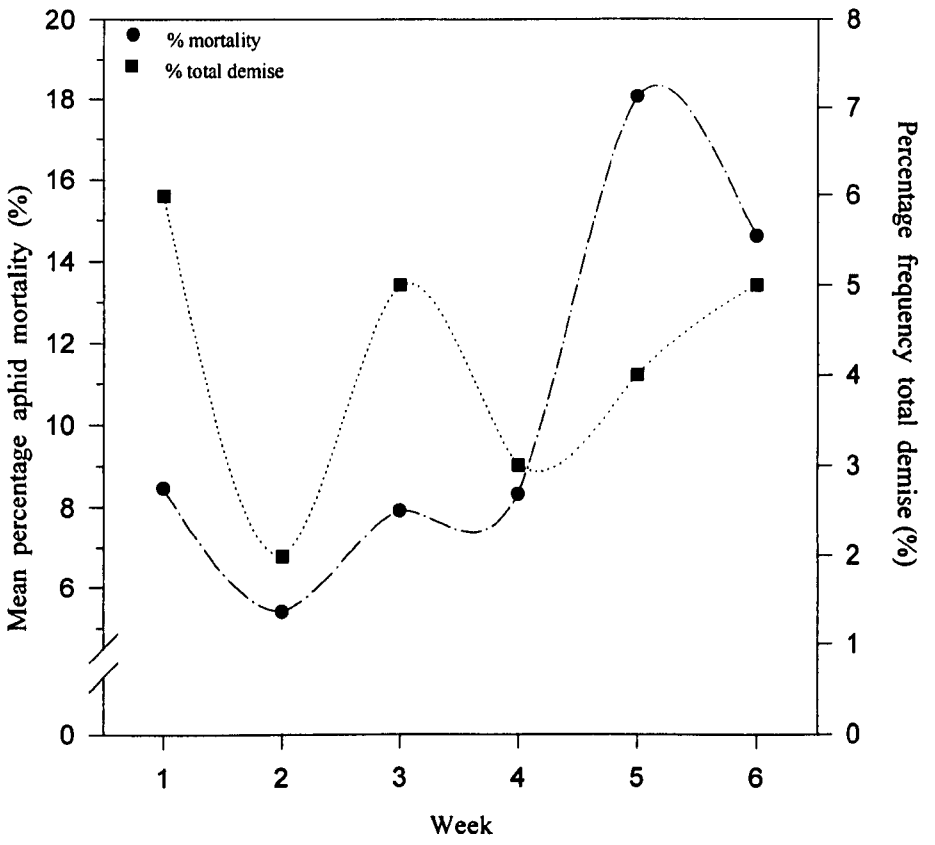
Relationship between wingless and alate aphid density



As the number of juvenile *Pemphigus spyrothecae* increased within the single gall, more alates were being produced ($r^2 = 0.134$, $p < 0.001$, $n = 700$). As few adults were seen prior to week 4, only data for weeks 4-6 were used.

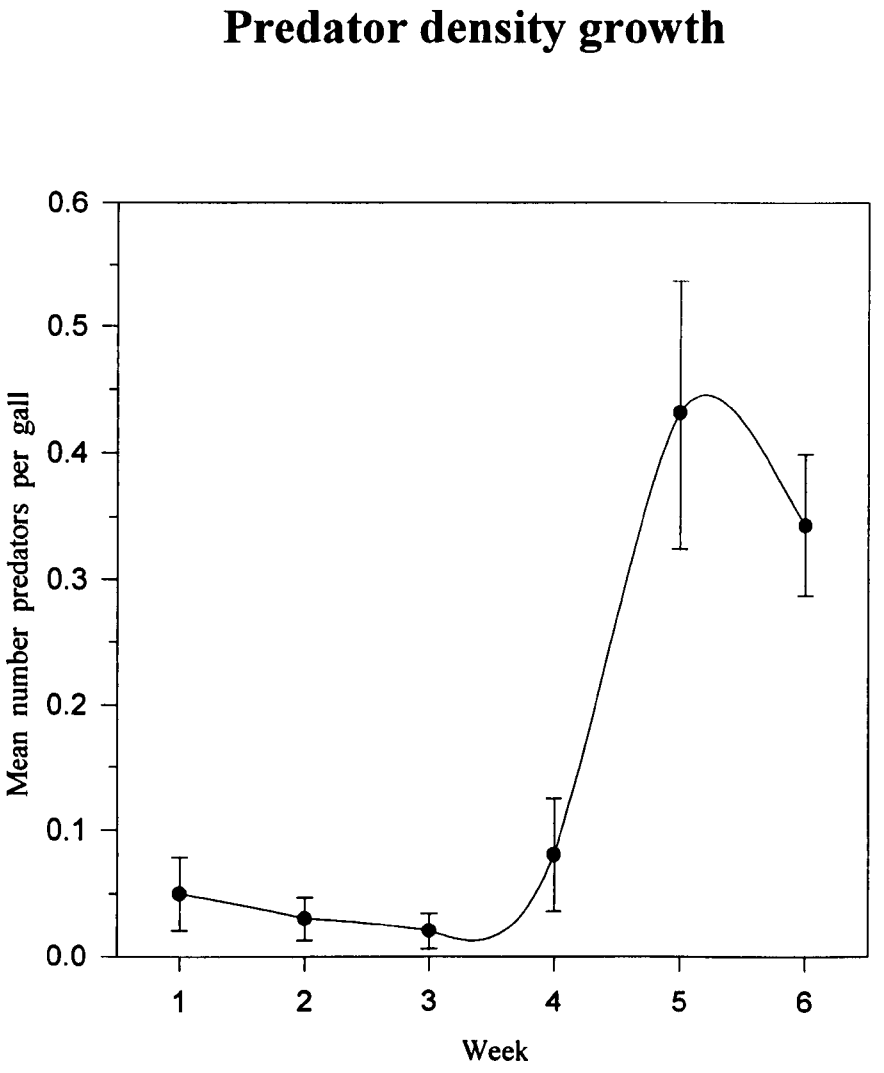
Figure 5. 3. 6

Aphid mortality during a six weeks study period



The percentage aphid mortality differed significantly among the six weeks starting 9th July (Kruskal-Wallis H = 260.36, $p < 0.0001$). The frequency of gall colony death did not appear to vary much during the study period (ranged between 2 and 6 %). Weeks 1-5: n = 100; week 6: n = 500. Only single galls were included in this analysis.

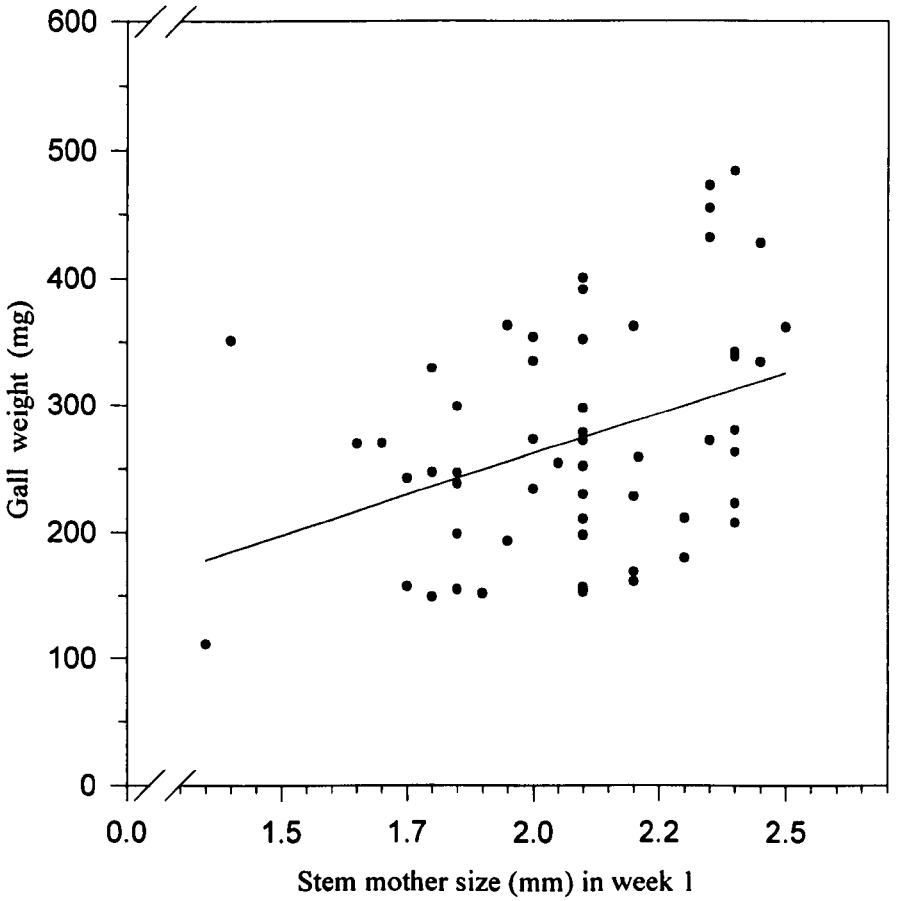
Figure 5. 3. 7



There was a significant change in the population density of *P. spyrothecae*'s main predator, *Anthocoris minki*, during the six week period starting 9th July (ANOVA $F_{5,999} = 5.23$, $p < 0.0001$). Weeks 1-5: n = 100; week 6: n = 500. Only single galls were included in this analysis.

Figure 5. 3. 8

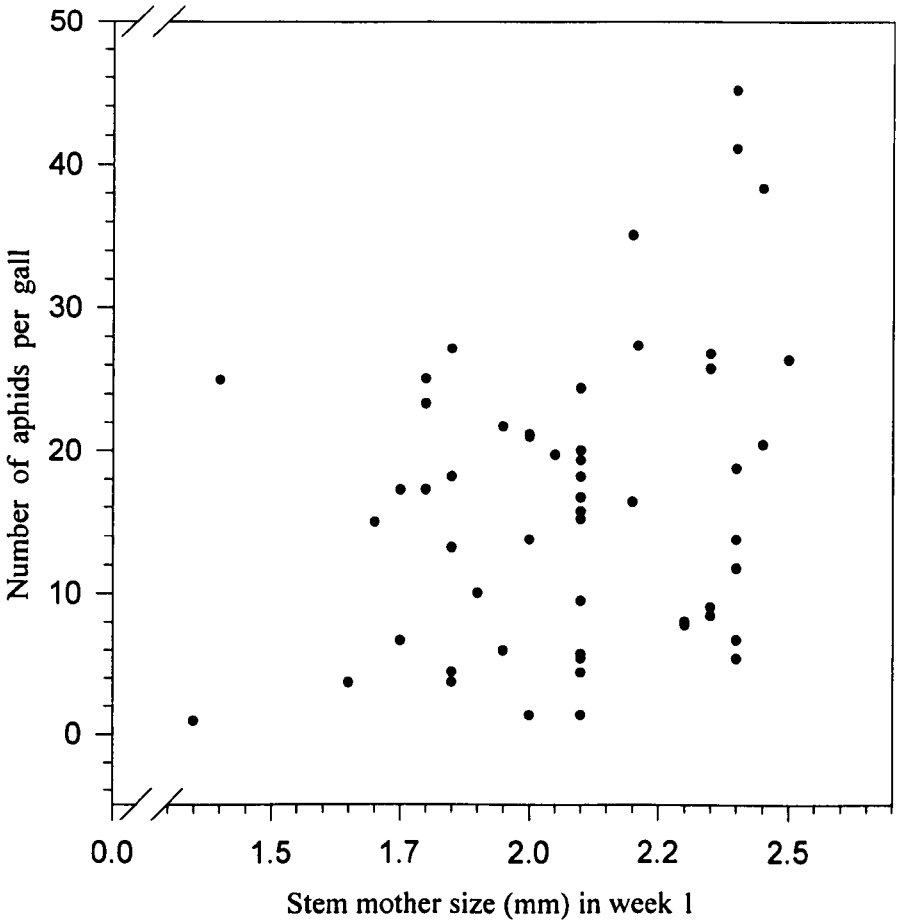
The effect of stem mother size on gall weight



In the first week commencing 9th July there was a significant relationship between *P. spyrothecae* stem mother size and gall weight ($r^2 = 0.13$, $p < 0.01$, $n = 55$). However, by the final week beginning 13th August, this relationship no longer existed ($r^2 = 0.013$, n.s., $n = 105$). Only single galls were included in this analysis.

Figure 5. 3. 9

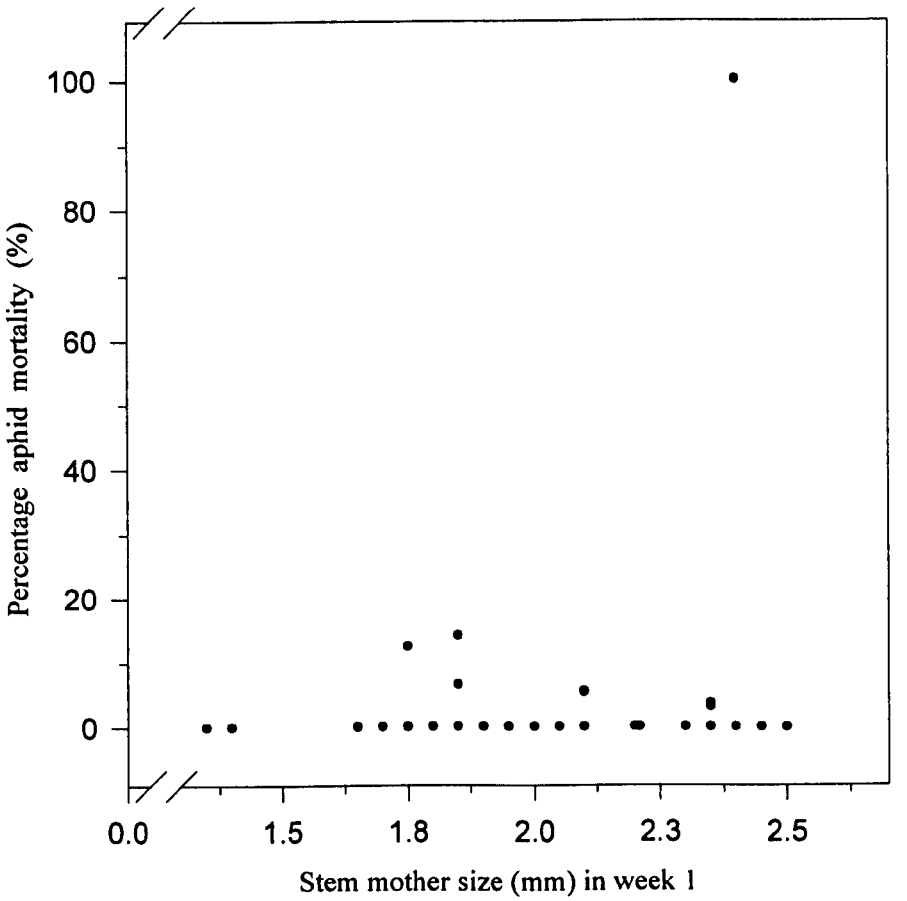
The effect of stem mother size on the number of aphids produced per gall



The size of *P. spyrothecae* stem mothers had no bearing on the number of aphids produced in the gall during the first week of study commencing 9th July (r^2 = 0.07, n.s., n = 52), nor in the final week (13th August) (r^2 = 0.03, n.s., n = 105). Only single galls were included in this analysis.

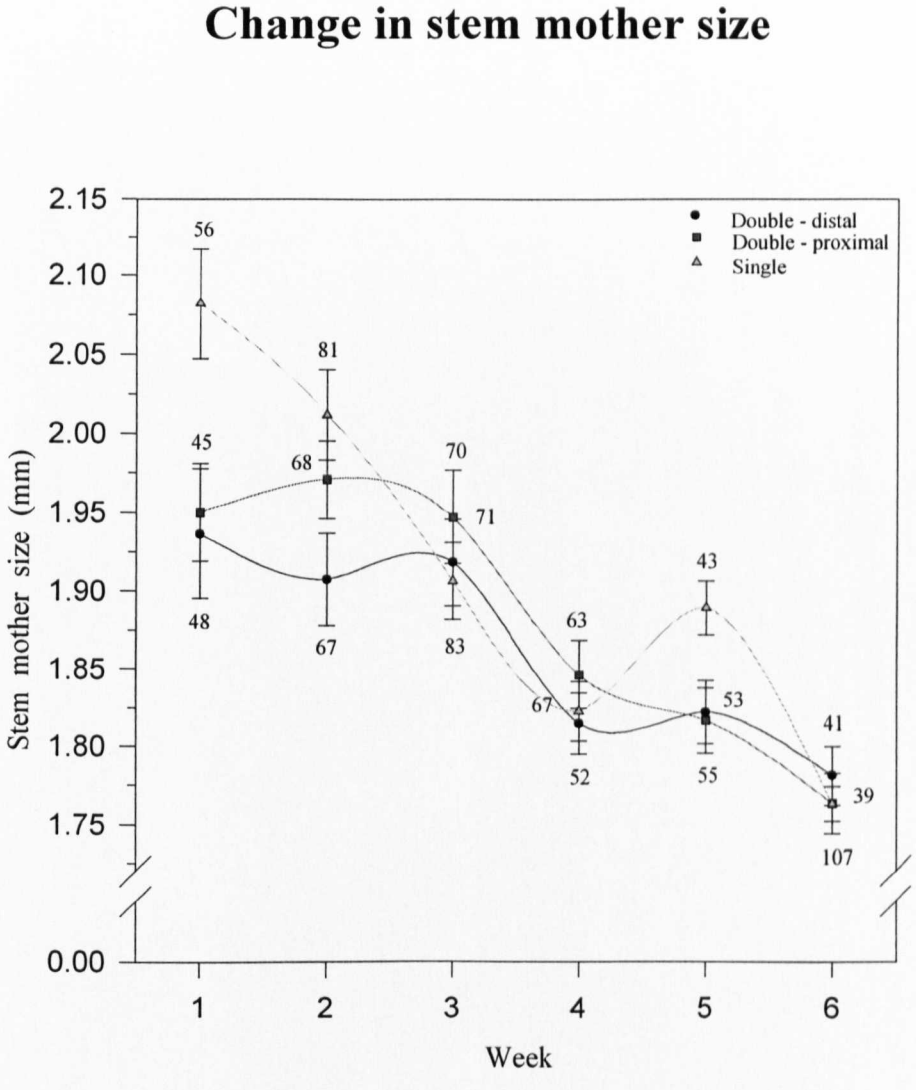
Figure 5. 3. 10

The effect of stem mother size on percentage aphid mortality



The size of *Pemphigus spyrothecae* stem mothers had no effect on the percentage aphid mortality in the first week commencing 9th July ($r^2 = 0.017$, n.s., n = 52) nor in the last week beginning 13th August ($r^2 = 0.018$, n.s., n = 105). Only single galls were included in this analysis.

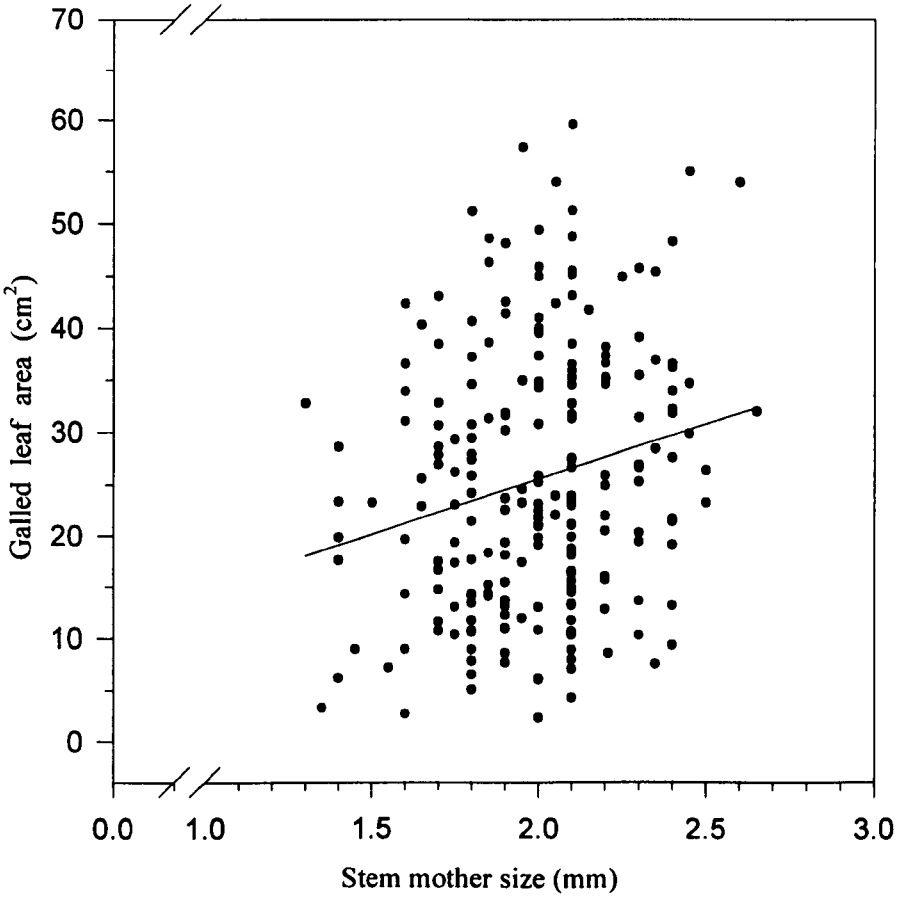
Figure 5. 3. 11



Pemphigus spyrothecae stem mothers differed in their average size during the six week period commencing 9th July (double stem mother distal to leaf: ANOVA $F_{5,331} = 4.94$, $p < 0.001$, double stem mother proximal to leaf: $F_{5,339} = 9.93$, $p < 0.0001$, and single stem mothers: $F_{5,436} = 27.35$, $p < 0.0001$). Generally, the size of the average stem mother reduced over time.

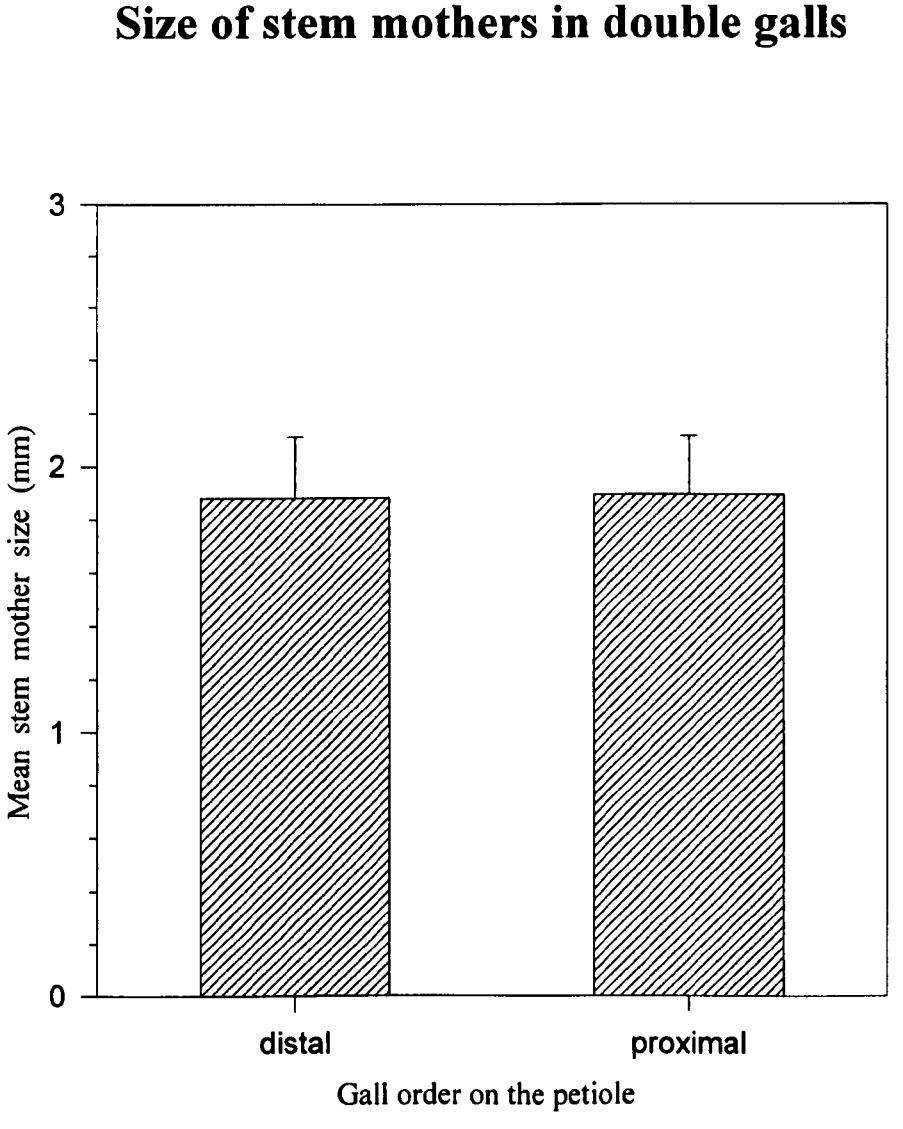
Figure 5. 3. 12

Stem mother size on different sized poplar leaves



Large stem mothers tended to occupy larger leaves than those colonised by smaller stem mothers ($r^2 = 0.046$, $p < 0.01$, $n = 218$). As stem mother size decreased with time (see Fig. 5.3.11) only data from weeks 1-3 were included in this analysis.

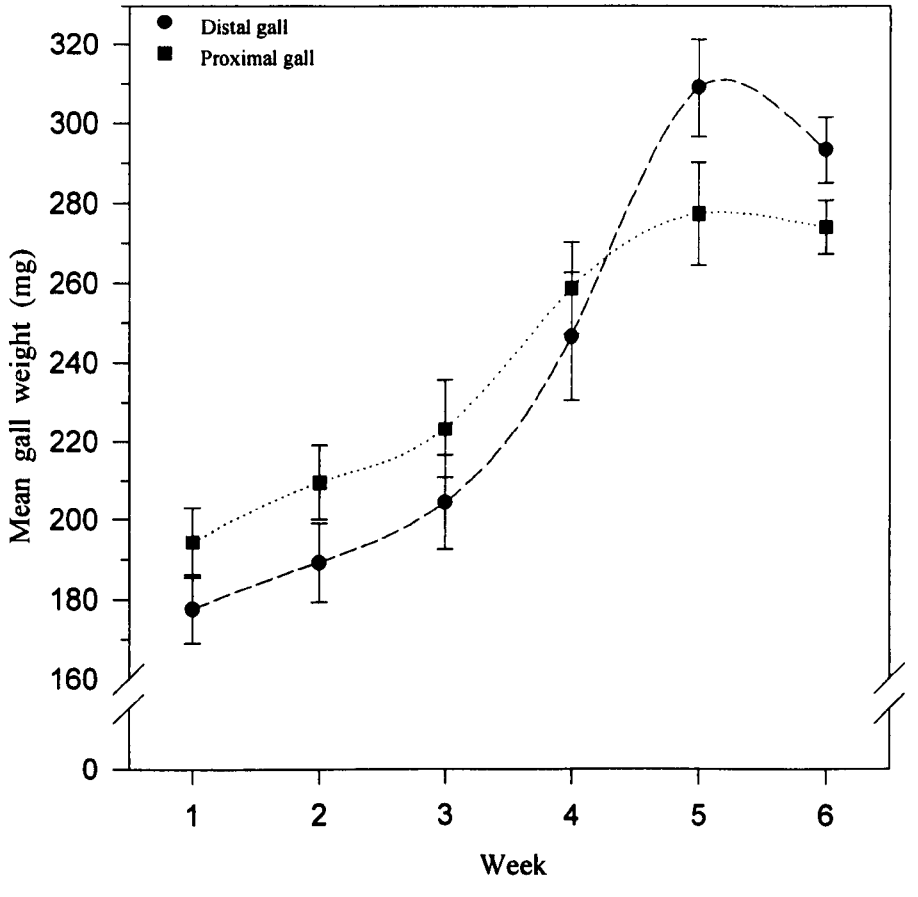
Figure 5. 3. 13



Pemphigus spyrothecae fundatrices sharing a petiole did not differ in size (matched paired t-test = -0.76, n.s., n = 187). The distal gall is furthest from the leaf. As stem mother size decreased with time (see Fig. 5.3.11) only data from weeks 1-3 were included in this analysis.

Figure 5. 3. 14

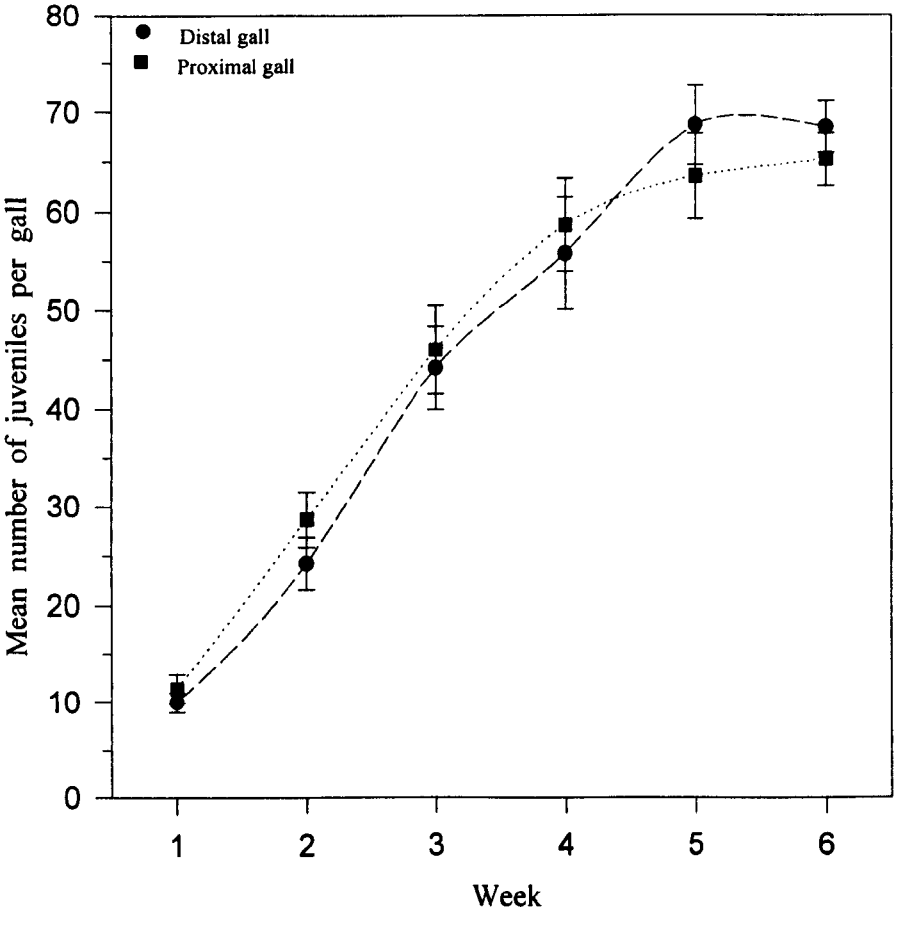
Growth in gall weights of double *P. spyrothecae* galls



During the six week study period commencing 9th July, with the exception of weeks 5 and 6, the average proximal gall (closest to the leaf) was heavier than the distal gall (furthest from the leaf). There were significant effects of gall order on gall weight during the six week period beginning 9th July (ANOVA: $F_{5,749} = 3.57$, $p < 0.01$) as a paired design.

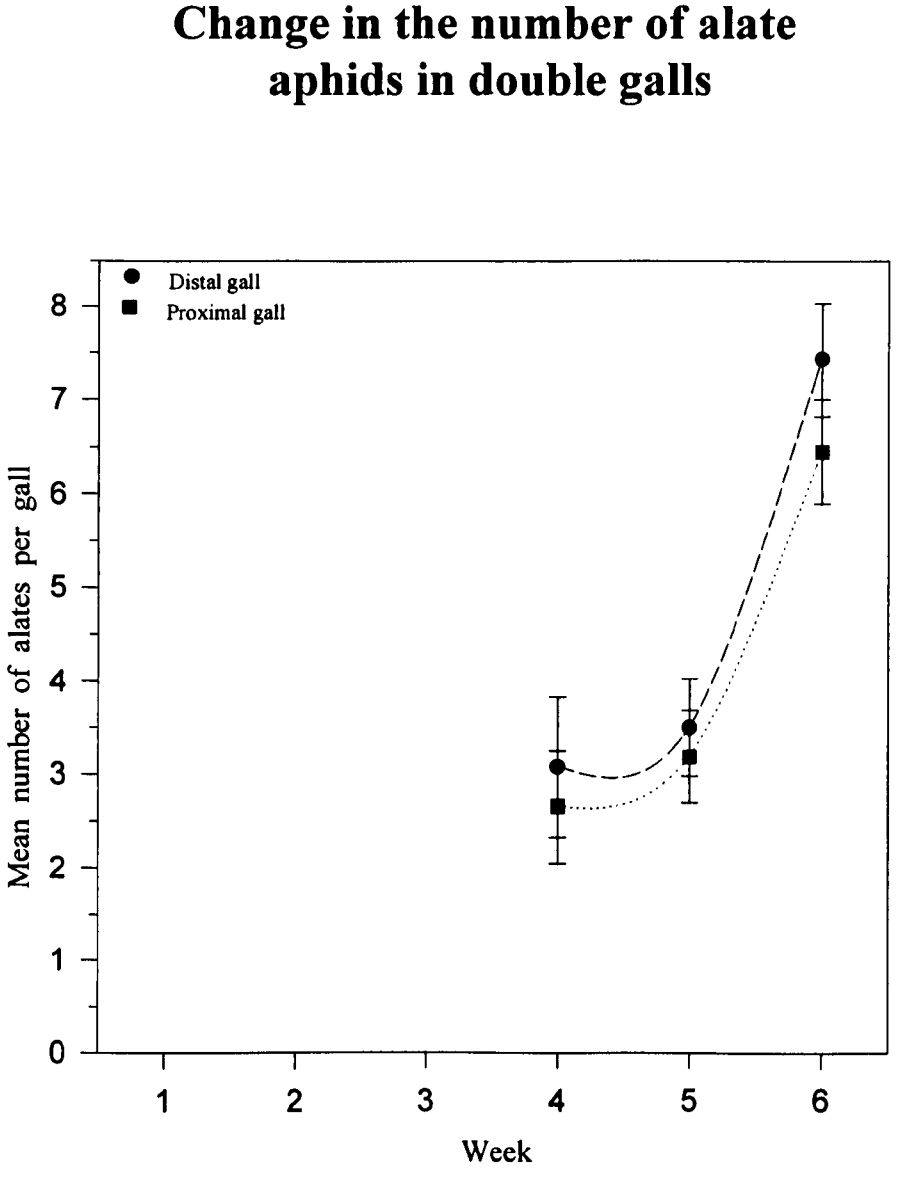
Figure 5. 3. 15

Change in the number of juvenile aphids in double galls



The number of *P. spyrothecae* wingless aphids in both the double galls grew steadily during the six week period beginning 9th July. There were no effects of gall order (proximal gall = closest to the leaf) on the number of wingless aphids during this period of growth (ANOVA: $F_{5,749} = 0.73$, n.s.) as a paired design.

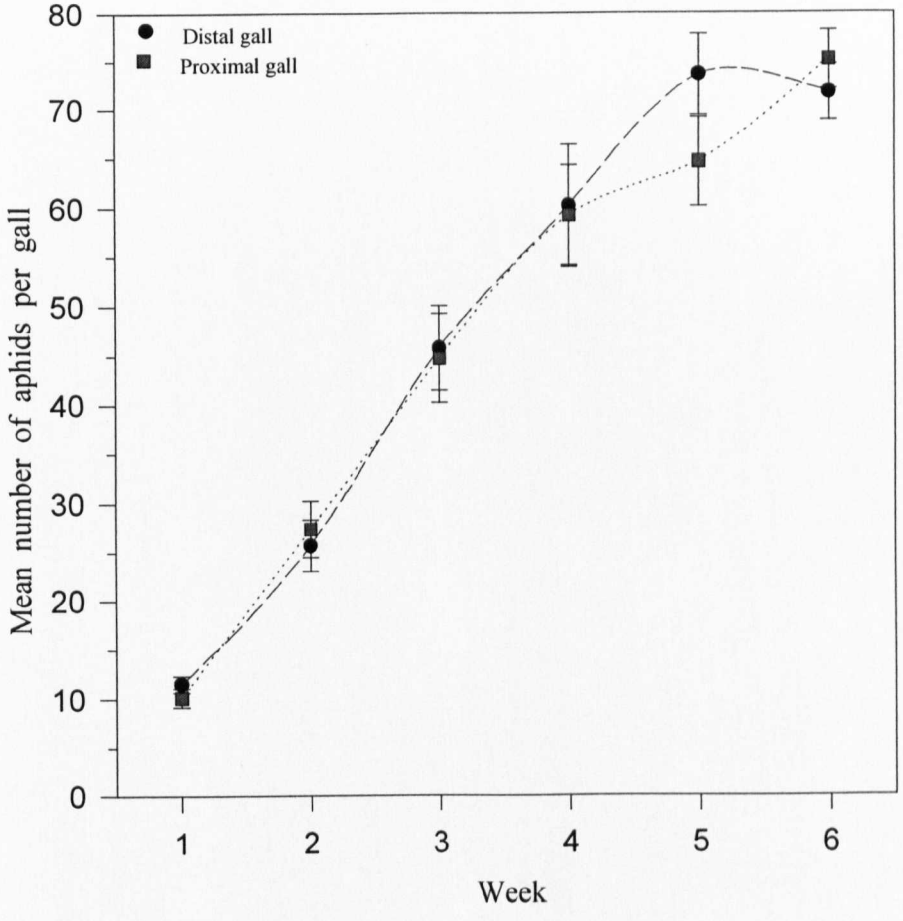
Figure 5. 3. 16



P. spyrothecae alate production commenced in the fourth week (30th July), and there did not appear to be any effects of gall order (distal = furthest from leaf) on the growth in the number of alates produced per gall (ANOVA: $F_{2,449} = 0.21$, n.s.) .

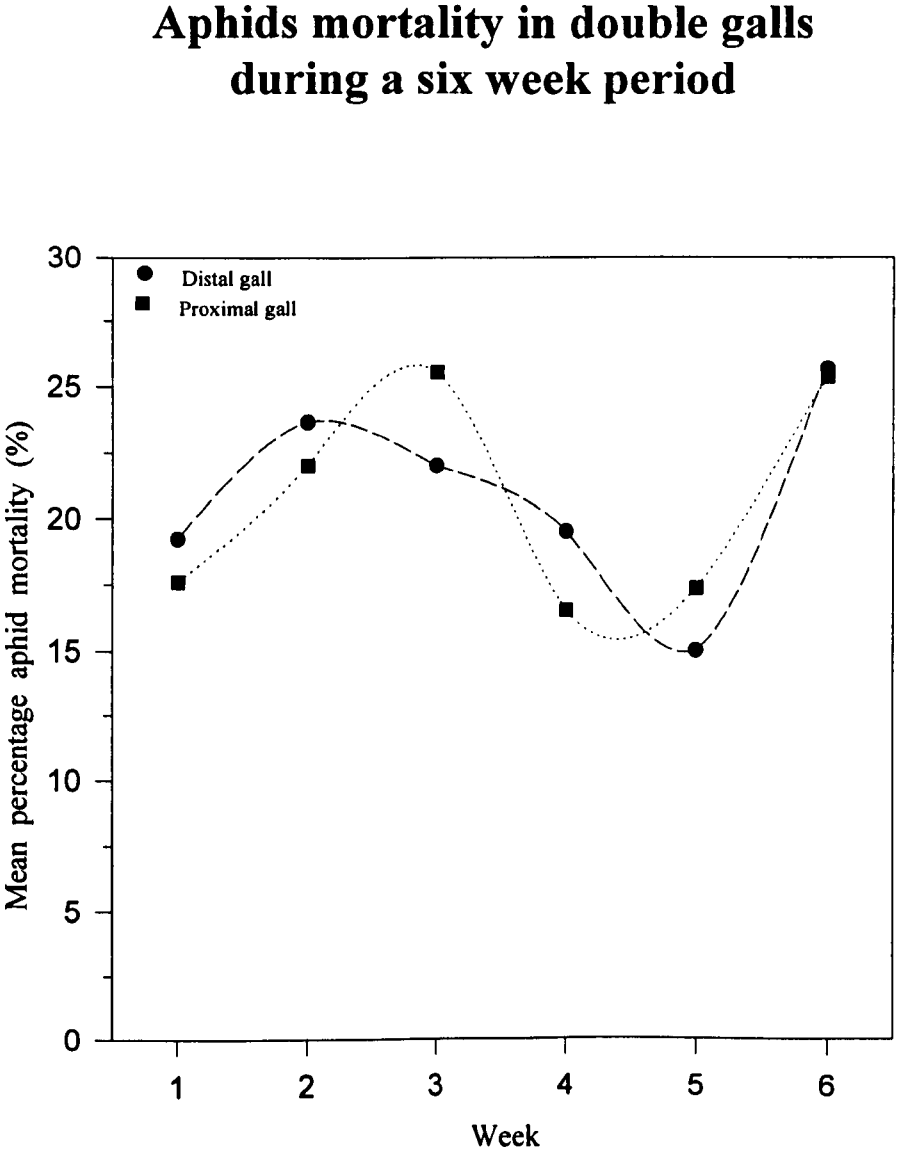
Figure 5. 3. 17

Change in the number of aphids in double galls



There was a steady growth in the number of aphids occupying both distal galls (furthest from the leaf) and proximal galls (closest to the leaf) during a six week period starting 9th July. There were no significant effects of gall order on the number of aphids per gall during the six week growth period (ANOVA: $F_{5,749} = 0.86$, n.s.).

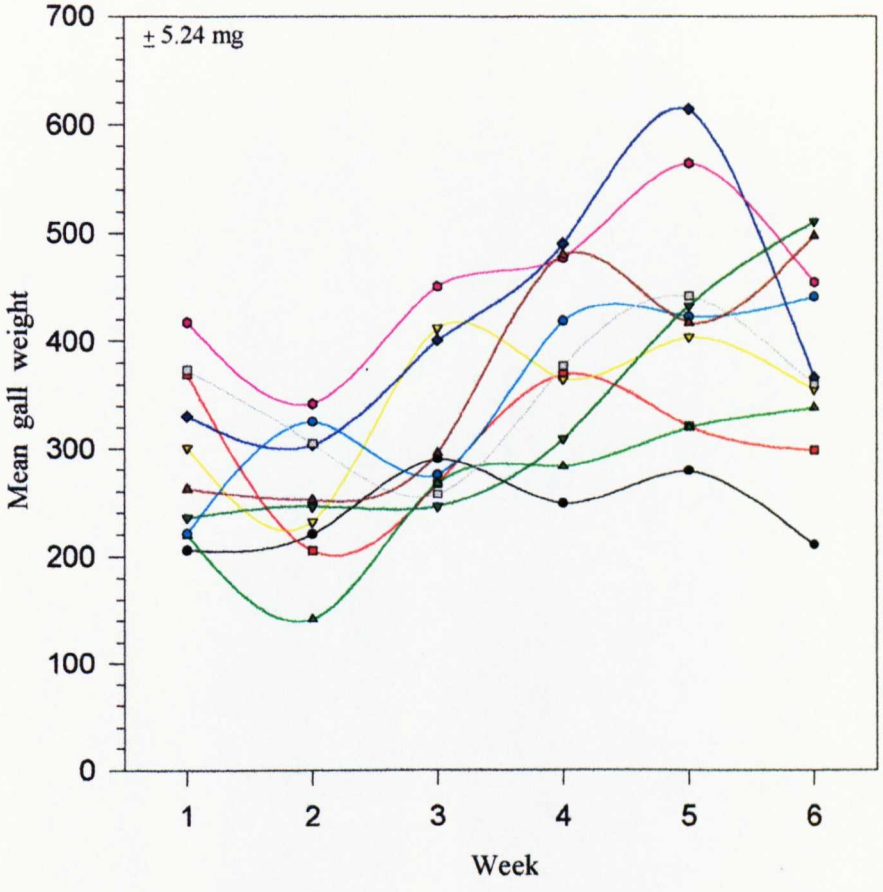
Figure 5. 3. 18



The average percentage mortality of *P. spyrothecae* aphids varied between 15-25%. (The proximal gall is closest to leaf). There were no significant effects of gall order on the percentage aphid mortality during the six weeks commencing 9th July (Kruskal-Wallis: $H_{5,749} = 1.04$, n.s.)

Figure 5. 3. 19

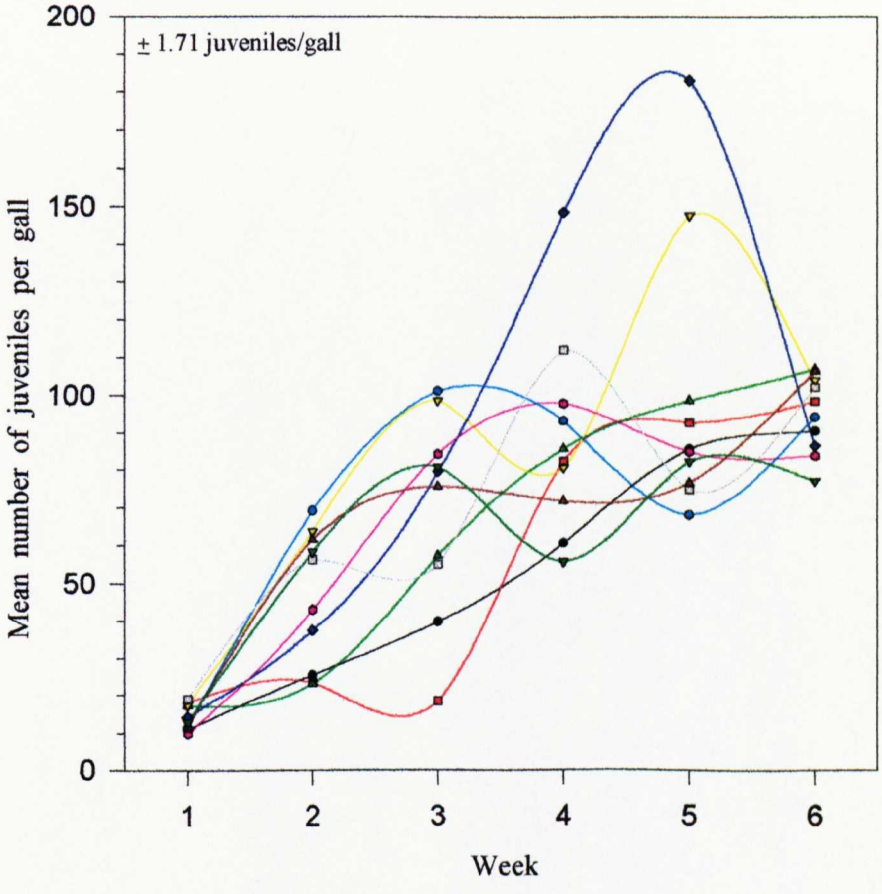
Change in gall weights among 10 poplar trees during a six week period



Each line represents one poplar tree. There were significant effects of trees and weeks on *Pemphigus spyrothecae* gall weights (2-way ANOVA: $F_{9,999} = 7.94$, $p < 0.001$ and $F_{5,999} = 69.05$, $p < 0.001$, respectively) and a significant interaction between weeks and tree ($F_{45,999} = 1.930$, $p < 0.001$) on gall weight. Commencing 9th July weeks 1-5: n = 100; week 6: n = 500. Only single galls were included in this analysis.

Figure 5. 3. 20

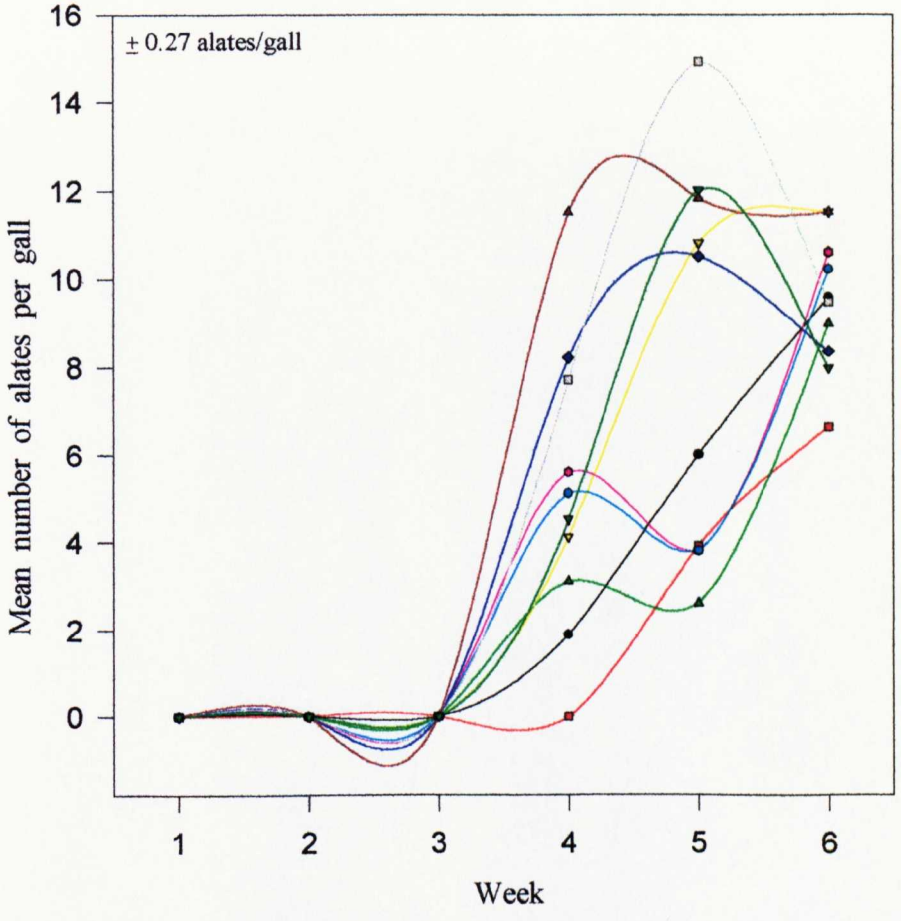
Changes in the number of wingless aphids per gall among 10 *Populus* trees during a six week study period



Each line represents one poplar tree. There were significant effects of trees and weeks on the number of *Pemphigus* wingless aphids per single gall (2-way ANOVA: $F_{9,999} = 5.16$, $p < 0.001$ and $F_{5,999} = 71.94$, $p < 0.001$, respectively) and a significant interaction between trees and weeks ($F_{45,999} = 2.88$, $p < 0.001$), indicating that the change in the number of wingless aphids per gall during the six week period differed among trees. Commencing 9th July weeks 1-5: n = 100; week 6: n = 500.

Figure 5. 3. 21

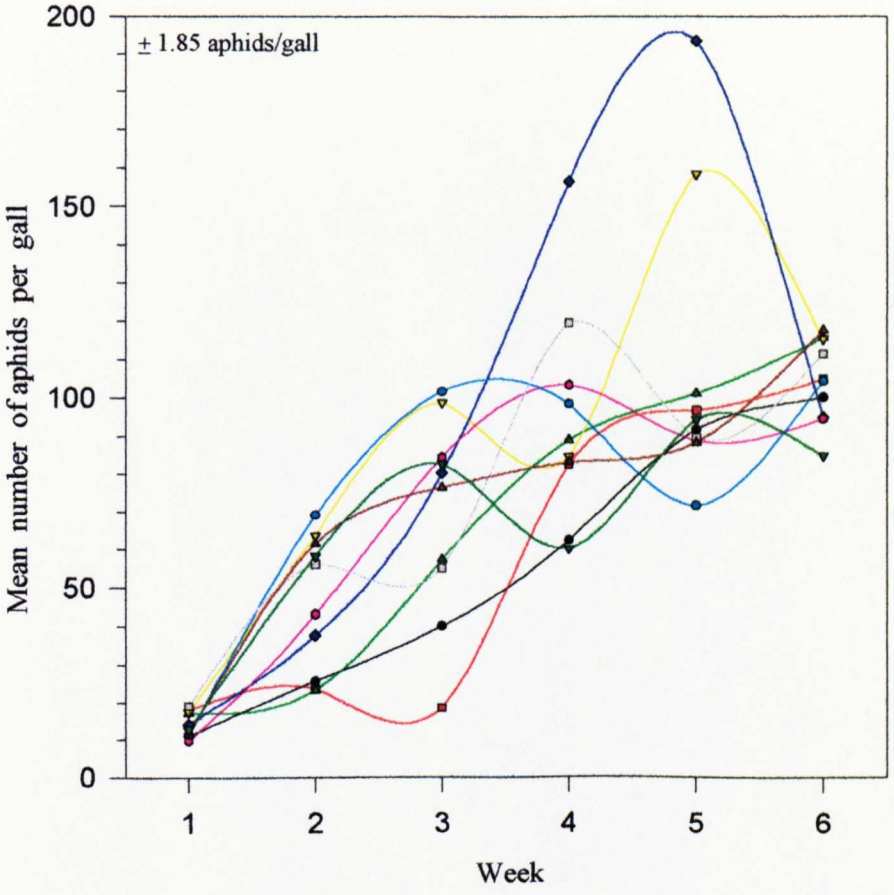
Changes in the number of alates per gall among poplar trees during a period of six weeks



Each line represents one poplar tree. Alate *Pemphigus spyrothecae* aphids began to appear in week four (30th July). There were significant effects of trees and weeks on the number of *Pemphigus* alates per single gall (2-way ANOVA: $F_{9,999} = 2.12$, $p < 0.001$ and $F_{5,999} = 58.86$, $p < 0.001$, respectively), but there was no interaction between trees and weeks ($F_{45,999} = 0.986$, n.s.) on the number of adult aphids per gall. Commencing 9th July weeks 1-5: n = 100; week 6: n = 500.

Figure 5. 3. 22

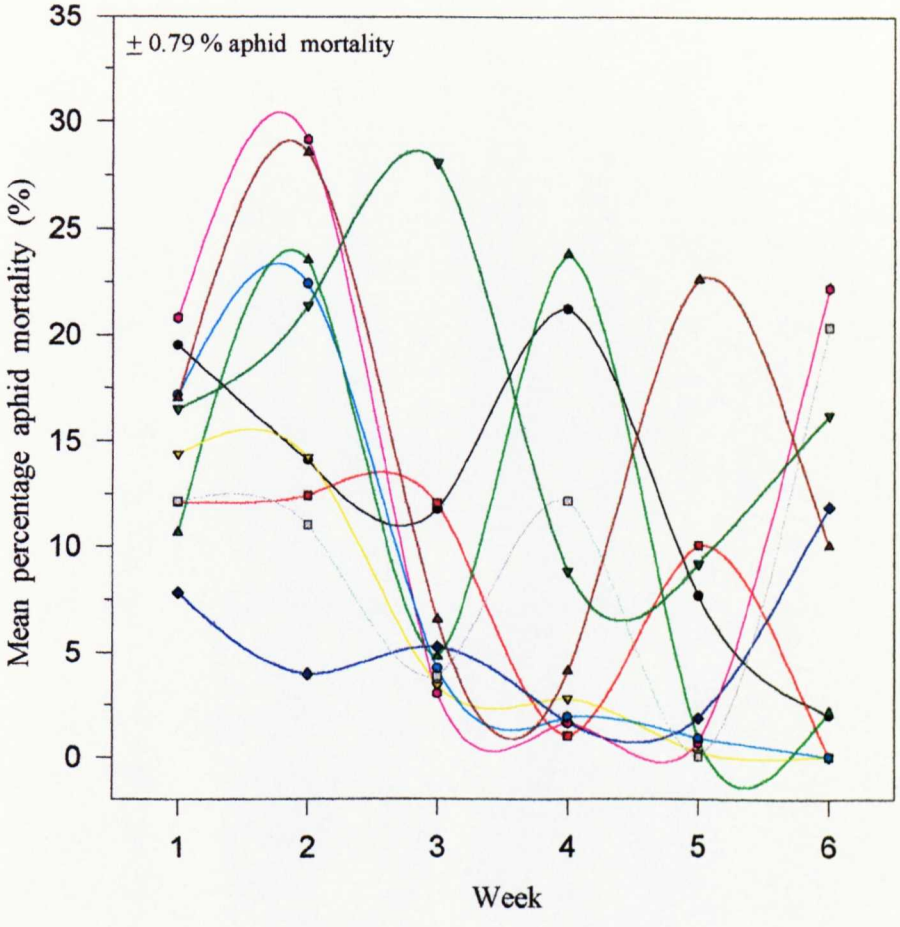
Change in the number of aphids per gall among poplar trees during a six week study period



Each line represents one poplar tree. There were significant effects of trees and weeks on the number of *Pemphigus spyrothecae* aphids per single gall (2-way ANOVA: $F_{9,999} = 5.01$, $p < 0.001$ and $F_{5,999} = 80.85$, $p < 0.001$, respectively), and there was also a significant interaction between trees and weeks ($F_{45,999} = 2.604$, $p < 0.001$), indicating that the change in the number of *P. spyrothecae* aphids per gall differed among trees. Commencing 9th July weeks 1-5: n = 100; week 6: n = 500.

Figure 5. 3. 23

Change in percentage aphid mortality among 10 poplar trees during a six week period



Each line represents one poplar tree. The percentage *Pemphigus* mortality did not differ significantly among the *Populus nigra* var *italica* trees in any week (weeks 1-6: Kruskal - Wallis H = 3.369, n.s.; H = 2.131, n.s.; H = 9.748, n.s.; H = 6.707, n.s.; H = 7.182, n.s. and H = 4.453, n.s., respectively). Commencing 9th July weeks 1-5: n = 100; week 6: n = 500. Only single galls were included in this analysis.

Chapter 6

THE EFFECTS OF MULTIPLY GALLED PETIOLES ON APHID FITNESS

6. 1 Introduction

Many studies have documented the occurrence of intraspecific competition in herbivores (e.g. Whitham, 1978, 1986; Quiring & McNeil, 1984; Craig *et al*, 1990). Individuals choose habitats that best suit their survival and reproductive success (Kimberling & Price, 1996) and if resources are limited, some individuals will be more successful than others. Competitive interactions commonly result in a differential fitness in survival and fecundity within a habitat. The mechanisms of competitive interactions vary among species. For instance, density-dependent larval mortality is caused by interference and exploitation competition in populations of the alfalfa blotch leafminer, *Agromyza frontella* (Quiring & McNeil, 1984), and larval survival is positively related to shoot length in the sawfly *Euura lasiolepsis* where females compete for high quality oviposition sites (Craig *et al*, 1990). Compared with galls occupying leaves singly, *Pemphigus betae* fundatrices doubling up on the same leaf suffer a reduction of 53% in the number of offspring produced (Whitham, 1983).

Galling insects are particularly vulnerable to the effects of competition because they are unable to avoid nearby individuals, and once a feeding site is selected, the individual remains there for the duration of its life. *P. betae* aphids prefer to locate their galls at the base of the largest *Populus angustifolia* leaf. This results in the highest production of progeny and the lowest mortality rate (Whitham, 1978). These leaves contain the lowest concentration of phenolics (Zucker, 1982) and act as effective resource sinks (Way & Cammell, 1970; Larson & Whitham, 1991). Although the evidence suggests there is strong selection pressure upon stem mothers to settle on large leaves, these preferred leaves are relatively rare (Whitham, 1978): although interference competition for food, especially the defence of feeding sites, is relatively unusual in insects, some species of aphids have been recorded fighting for access to choice plant tissue (Foster, 1996; Aoki & Kurosu, 1985). Fighting has also

been observed in the Pemphigidae (e.g. Whitham, 1979; Aoki & Makino, 1982): for instance, first-instar *Pemphigus betae* foundresses compete for superior galling sites on developing leaves (Whitham, 1978).

The main prediction of this study was that competition for nutrients would lead to a decrease in fitness components. I looked for the possible effects of intraspecific competition in *Pemphigus spyrothecae* aphids using both observational data and experimental manipulation. The observational data consisted of 1) a comparison of the locations of single and double galls on the host *Populus* x *canadensis*, within a shoot and on a single petiole; 2) the effects of the distance between double galls on a single petiole; 3) the effects of gall order along the petiole and 4) a direct comparison of reproductive success between single and double galls. By manipulating the number of successful galls per petiole, I tested the hypothesis that the removal of a competitor would facilitate reproductive release.

6. 2 Methods

The life cycle of *Pemphigus spyrothecae* Passerini, 1860 has been described by Dunn (1966) and Foster (1990). This species is not host alternating, but spends its whole life cycle on its primary host, the black poplar *Populus nigra* and its hybrids and varieties. Eggs laid the previous year in the bark on branches or on the trunk of the host tree, hatch in spring and produce parthenogenetic stem mothers (first generation). The wingless stem mothers are very mobile, and migrate out to the newly emerged shoots, where they select a petiole for gall formation. By midsummer up to several hundred offspring are parthenogenetically produced. The first instars grow into wingless adults (virginoparae, second generation), and these in turn produce both thick-legged and normal-legged first instars. The normal-legged instars grow into winged sexuparae (third generation), that, once the galls have opened in August, migrate to the trunk, give birth to sexuals (fourth generation), which mate and produce overwintering eggs in the bark of the tree.

The hybrid black poplar (*Populus* x *canadensis*) trees used in this study are located at Attenborough Gravel Pits, Nottingham, England, a Site of Special Scientific Interest (SSSI), jointly managed by Nottinghamshire Wildlife Trust and owners Butterley Aggregates Ltd. Mature trees were used, as juvenile trees are resistant to galling and are avoided by migrants (Kearsley & Whitham, 1989). The trees grow close together along a stream near a disused car park, and hence are all subjected to similar climatic and environmental conditions. The trees were planted approximately 25 years ago.

A total of approximately 400 galled shoots were unbiasedly collected prior to gall opening in early August from eight (A-H) hybrid black poplar trees. These were placed in a freezer at -20° C to prevent the final development and release of the alates (winged progeny). For ease I selected shoots from the lower 3 metres of each tree. For the purpose of this study, a shoot is defined as the product of a single season's growth from a single bud (Fay & Whitham 1990). Gall distance to the tree trunk was measured to the nearest half a metre. The position of leaves on the shoot was recorded by ranking them from the apical leaf. The size of individual galled leaves was later measured using the greatest length and width in mm. Leaf area was calculated by

placing the leaf under a 1 cm^2 transparent grid, and summing the number of 1 cm^2 units within the leaf outlines. Petiole length was measured in mm and the position of the gall on the petiole was calculated, with 0% being at the shoot junction and 100% at the leaf junction. Competitor density was measured as the number of galls per petiole. Gall failures were classified as those fundatrices that did not initiate a successful gall, seen later as a kink in the petiole, or those that died before gall completion, forming an undeveloped empty gall. If two fundatrices move onto the same petiole, they may both initiate galls, but one or the other may leave before gall completion (Dunn, 1960).

In order to ascertain how reproductive success can be manipulated in a competitive situation, I matched three galls from individual petioles. The first was a single gall, and the second was a distal gall (furthest from the leaf) on a doubly-galled petiole, where the stem mother from the proximal gall had been removed after gall initiation in spring. The third gall was a distal gall on a doubly-galled petiole but with an intact proximal gall. The three galls were matched for leaf and shoot size, and for location on the shoot. 6, 8 and 5 sets were collected from trees C, D and E, respectively.

Individual galls were weighed using a Cahn electro-balance. Each gall was opened and the contents gently transferred with a paintbrush into a petridish containing 70% methcol and the number of aphids and winged progeny counted under a Wild dissecting microscope.

Sample sizes and standard errors are included where possible in graphs showing mean values. The data were checked for normality, and where necessary, non-parametric statistics were used for analyses.

6. 3 Results

The problem of selecting an appropriate site for gall formation is complex because a stem mother has a choice between making a gall on an occupied petiole or migrating to another shoot or leaf which may not be as suitable. Doubly-galled poplar petioles were, on average, located further from the tree trunk than single galls (Fig. 6.3.1). Of the 82 cases where a shoot had two *P. spyrothecae* galls, 85% were located on separate petioles. Doubly-galled petioles were most often found sharing a shoot with a single gall (Fig. 6.3.2). When comparing shoots with either one singly-galled or one doubly-galled petiole, both petioles occupied, on average, similar positions on shoots with the same number of leaves (Fig. 6.3.3). Likewise, there was no difference in petiole length or size of the biggest leaf on the shoot (Fig. 6.3.4).

When a doubly-galled petiole shared a shoot with a single gall, the double galls were, on average, closer to the shoot apex than the single gall, but this was not statistically significant (Fig. 6.3.5). Single and double galls occupied leaves of similar length and width (Fig. 6.3.6), and although doubly-galled petioles had, on average, a larger area of leaf tissue, there was no apparent statistical difference (Fig. 6.3.7).

The mean distance between double galls on the same petiole was less than 12 mm, and, in general, greater distances between the galls resulted in heavier galls (Fig. 6.3.8) containing more aphids (Fig. 6.3.9). Distance between double galls had no apparent effect on the probability of gall failure (Fig. 6.3.10). I predicted that stem mothers who initiated their gall on a petiole already occupied by another would run a higher risk of failure. However, this was not the case ($\chi^2_2 = 1.78$, n.s.), since 74% of all aborted galls were alone on the petiole. Just 9% of distal (the gall furthest from the leaf) galls and 17% of proximal galls on doubly-galled petioles were gall failures.

The order of galls on the petiole of doubly-galled leaves had a significant effect on gall weight with the distal (the gall furthest from the leaf) weighing more than the proximal gall (Fig. 6.3.11). Similarly, the galls furthest from the leaf had, on average, more offspring than those closest to the leaf, but the difference between these means was not statistically significant (Fig. 6.3.12). However, when comparing singly with doubly-

galled petioles there was a clear reduction in gall weight (Fig. 6.3.13) and reproductive success (Fig. 6.3.14), but this had no effect on percentage gall failure (Fig. 6.3.15).

In the removal experiment I matched a single *P. spyrothecae* gall with two distal galls (furthest from the leaf) from two doubly-galled *Populus* x *canadensis* petioles. On one of the doubly-galled petioles, the fundatrix from the proximal gall (closest to the leaf) was removed shortly after gall initiation in spring. Mean gall weights and reproductive success of single galls were greater than both double galls. However, the distal galls without their partner proximal galls were, on average, heavier and contained more offspring than the distal galls on the intact doubly-galled petioles. Although there were no apparent statistical effects on gall weights across categories (Fig. 6.3.16), reproductive successes were significantly different (Fig. 6.3.17), but a post-hoc comparison between the removed and the double categories was not significant.

Fig. 6.3.18 and 6.3.19 illustate how *P. spyrothecae* aphids performed under different competitor densities and habitat qualities. Both gall weight and the number of aphids per gall increased as habitat quality (leaf size) increased, but, if leaf size was held constant, decreased with increased competitor density. Overall, gall density had a significant effect on both gall weight and reproductive success.

6.4 Discussion

The presence of other individuals is the main factor which often affects which part of the habitat provides the most suitable conditions to maximise fitnes. Insects influence each others success through various density dependent processes. These include exploitation competition, where the quantity of available resources is reduced; interference where fitness is reduced by factors such as wasting time in interactions with other foragers; and the influence of density on predation risk. Insects are not only distributed in relation to their required resources, but also with respect to their competitors (Treganza, 1995).

The distribution pattern of *Pemphigus spyrothecae* stem mothers on petioles of the hybrid black poplar *Populus* x *canadensis* revealed that stem mothers are more likely to double up on petioles located further from the tree trunk. Bigger shoots are found on the distal branches furthest from the trunk, and stem mothers are risking a longer migration to these bigger shoots which may be nutritionally superior, even if this means sharing a shoot with another individual. Some studies show that aggregation can improve the food available to the newly emerged nymphs by acting as physiological sinks for photosynthate originating elsewhere in the plant (e.g. Way & Cammell, 1970; Heard & Buchanan, 1998). However, although aggregation initially enhances multiplication (Way, 1968; Way & Banks, 1967), the rate is quickly slowed as the numbers in the aggregate increase above a relatively small critical level, restricting the rate of increase, and inducing early leaf abscission which reduces survival compared to solitary aphids (Williams & Whitham, 1986). In this study doubly-galled petioles frequently shared a shoot with other *P. spyrothecae* galls, indicating that strong competitive interference is likely.

Both single and double galls occupied shoots, leaves and petioles of a similar size and although doubly-galled petioles were, on average, further from the shoot apex, there was no apparent significant effect. I would have expected leaf or shoot size to be larger for double galls than single galls, because these shoots and leaves must support significantly larger quantities of aphids. However, my recent findings suggest that position on a shoot is not important to *P. spyrothecae* aphids because they successfully act as physiological sinks by drawing resources from neighbouring leaf

tissue. Other studies (e.g. Ngakan & Yukawa, 1996; Wool & Bar-El, 1995) suggest that certain positions on a shoot are more popular than others. For instance, the distribution of *Forda formicaria* galls on leaves was non-random, with an excess of both ungalled and crowded leaves (five galls or more) (Wool & Bar-El, 1995). Budburst synchrony may not only in part explain why doubly-galled petioles tended to be further from the shoot apex, but also why many leaves on shoots contained either no galls or more than one gall.

The effect of distance between two *Pemphigus spyrothecae* galls on the same petiole (if the data for 0-5mm are disregarded momentarily) suggests that if galls share the same petiole, they both benefit in terms of increased reproductive success from being located as far from each other as possible. When galls are positioned close together (0-5mm) they may act in unity as a single gall by increasing the flow of translocated resources. The order of the gall along the petiole was important, and this was consistent with Whitham's (1979) study which showed that if several *P. betae* aphids gall along the midrib of a leaf, the stem mother occupying the preferred basal position on the leaf achieved the greatest success. Galls distal to the leaf were significantly heavier, and tended, on average, to have more offspring suggesting that distal galls are usurping nutrients from the proximal gall. The ability of a gall to translocate assimilates and nutrients from neighbouring leaflets or leaves caused a detrimental effect on other galls sharing the same leaf (Burstein, Wool & Eshel, 1994).

Solitary *P. betae* stem mothers produced more progeny than stem mothers that shared leaves (Whitham, 1986). This is also the case for *P. spyrothecae* (see Figs. 6.3.13 and 14). Single galls were heavier and contained more offspring than either double or triple galls regardless of order on the petiole. However, there was no effect on gall failure (Fig. 6.3.15). Similarly, no difference in failure rates of *P. betae* stem mothers was found on leaves containing one, two, three or four galls per leaf (Whitham, 1980). In contrast, survival decreased when *Cynips divisa* gall density increased (Gilbert *et al*, 1994). A density increase had significant effects on *Cynips divisa* female weight and relative weight (mass for size), but not on allocation (proportion of body weight allocated to abdomen) or fecundity (number of eggs). Other examples of detrimental effects of competitive interference include Ngakan & Yukawa's (1996) study. As

Neothoracaphis yanonis gall density increased, gall size decreased, and the percentage of indehiscent galls increased. They surmise that the density effect promoted the dispersal of stem mothers whilst reducing fitness of aphids located on less favourable sites. In some circumstances, interference competition appears to be absent. For instance, intraspecific competition does not influence habitat selection of *Daktulosphaira vitifoliae* galling on the leaves of wild grape *Vitis arizonica* at the within-leaf spatial scale (Kimberley & Price, 1996). However, host quality was important to grape phylloxera fitness at greater scales.

For any competitor density, as *Populus* x *canadensis* leaf size increased, the average *Pemphigus spyrothecae* gall weight and number of progeny increased (Figs 6.3.18 and 19). However, at a constant habitat quality, average fitness decreased when competitor density increased. This suggests that *P. spyrothecae* aphids compete intraspecifically. In contrast to *P. betae* (Whitham, 1980), *P. spyrothecae* stem mothers have not adjusted their average fitness in the various leaf size categories such that the average fitness was equal, regardless of number of competitors. There was a significant effect of competitor density on the number of progeny and gall weight (reduced as competition increased) across all leaf size categories.

Pemphigus spyrothecae fundatrices occupying leaves singly from the beginning of gall formation produced more progeny than either member of a competing pair. When released from competition early in development (one member of pair removed) the remaining stem mothers produced, on average, smaller galls and fewer offspring than single galls, but more than double galls. Competing *P. betae* stem mothers spent less time feeding and/or probing leaf tissue, because they spent nearly all their time in aggressive interactions at the boundary between territories (Whitham, 1986). At budburst, leaves rapidly expand and with only immature leaf tissue susceptible to galling, aggressive interactions interrupt gall formation and reproduction for both competing individuals. Although larger basal stem mothers are more fecund, the basal position of the leaf blade is superior to distal sites and this explains the observed effect more than body size. Whitham (1979) conducted a removal experiment during stem mother colonisation which showed that on high density trees, stem mothers were moving in search of a place to settle and were reacting negatively to the presence of

other stem mothers, whereas on low density trees, stem mothers were rapidly able to select leaves and commence gall initiation.

Territorial contests between *P. betae* aphids at colonisation lasted up to two days and were determined by kicking and shoving fights (I observed a similar behaviour in *P. spyrothecae* fundatrices during a study conducted at budburst) with the largest stem mother usually winning (Whitham, 1979). If a basal stem mother was removed, the distal stem mother shifted downwards to the basal position. If contest time is evolutionarily determined, then contests lasting more than a few minutes suggest that great dividends or losses are at stake. 93% of larger aphids of the species *Astegopteryx minuta* won fights, with 74% of attackers larger than the defenders (Foster, 1996). There were no obvious wounds to participants, but mouthparts, antennae or horns may be injured in a fight. In another aphid, *Epipemphigus niisimae*, which forms cockscomb-like galls on the leaves of *Populus maximowiczii,* the fundatrix invades other conspecific galls and fights the owner often until death (Aoki & Makino, 1982). The first instar is a formidable fighter with enlarged forelegs and strongly sclerotized tergites. Fighting takes place using strikes by forelegs which may injure the opponent (e.g. aphids were found lacking antennae), but fatalities usually occur if stung with the mouthparts.

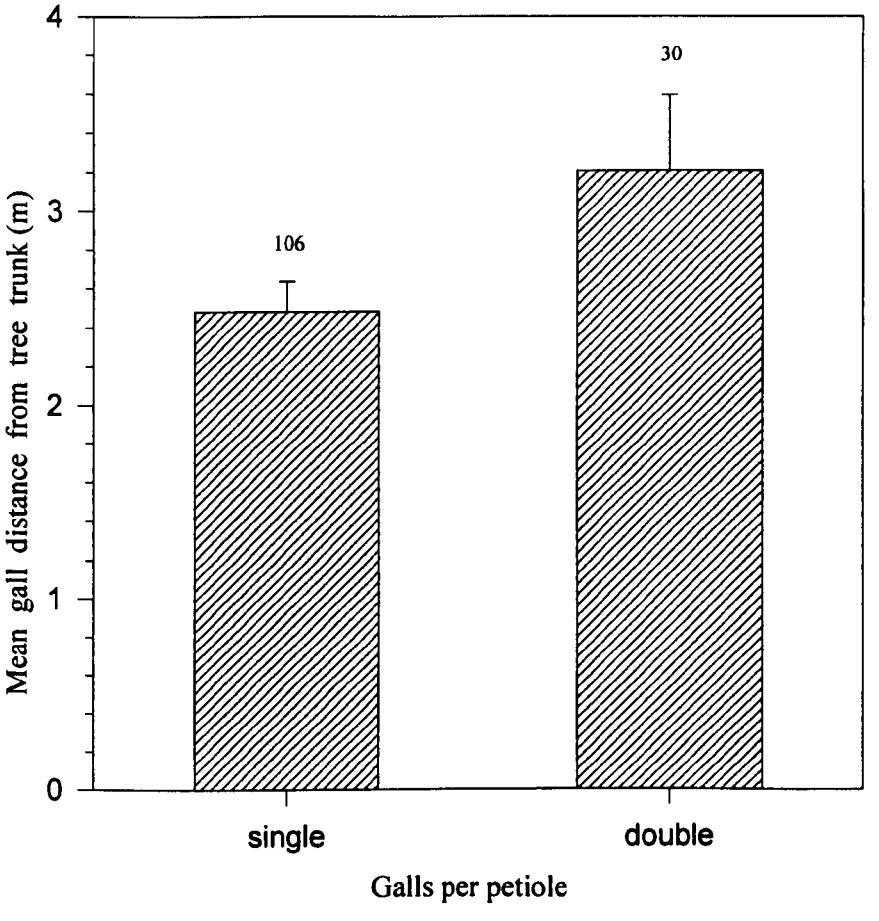
Some authors note (e.g. Foster, 1996; Aoki & Kurosu, 1985; Inbar *et al*, 1995) that aphids fighting for access to plant tissue may be either gaining time or improving the quality of a feeding site. Some aphids take a long time to find and puncture a sieve element from which they can obtain a sustained amount of resources, and thus a feeding site could be regarded as a territory. The winner will insert its stylet into the exact spot where the defender was feeding. So by fighting for a few minutes, aphids save many hours by taking possession of a sieve tube already punctured (Tjallingii, 1994). As already mentioned, single aphids and aphid aggregations are able to induce sinks for plant assimilates (Inbar *et al*, 1995), and a feeding site already used may perhaps be a better quality sink than one yet to be established.

Why *Pemphigus spyrothecae* aphids choose to double up on a petiole remains unclear, as the data revealed no apparent benefits from sharing a petiole. Doubling up is not a

common occurrence; of the 82 cases where a shoot had two galls, 85% were located on separate petioles. The galled leaf tissue area is very important to *P. betae* fitness (Whitham, 1979,1980), whereas my previous work (no effect of gall position on shoot) suggests that shoot area has a greater effect on *Pemphigus spyrothecae*'s reproductive success. Perhaps doubling up on the same petiole relieves the second stem mother from galling on unsuitable leaves on an otherwise appropriate shoot. Doubling up may reduce the risk of predation in *P. spyrothecae*, although the probability of being preyed upon did not differ for leaves with one or two *P. betae* galls (Whitham, 1980). Gall failure was not more common on shared petioles. Sharing a leaf is more likely to be an effect of budburst synchrony. Previous work suggests that only newly-formed closed leaves are suitable for colonisation. Unfortunately, an experiment conducted at the time of budburst (observing the colonising process of *P. spyrothecae* stem mothers along a poplar branch) did not manifest any doubly-galled petioles. It would have been useful to observe whether double galls are the result of stem mothers arriving on the petiole at different times; the first stem mother starts galling just prior to the second stem mother arriving, or whether fighting results in the losing stem mother being banished to a galling site closer to the leaf junction where the average fitness is lower.

Figure 6. 3. 1

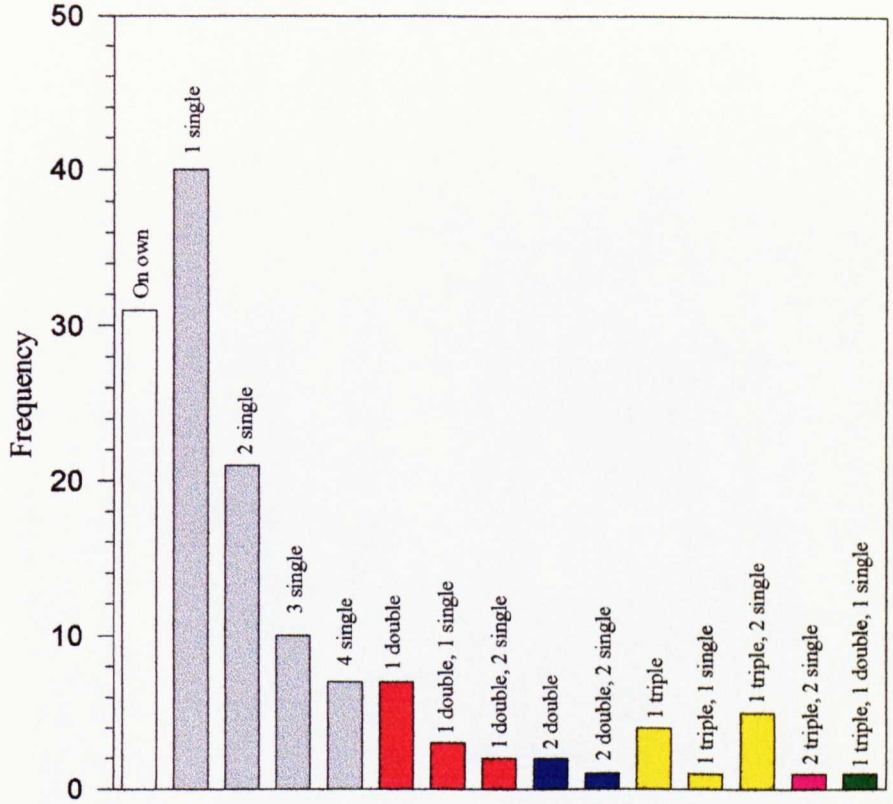
Comparison of single and double gall distance to the poplar tree trunk



Doubly-galled petioles are located further from the poplar tree trunk (ANOVA: $F_{1,135} = 3.98$, $p < 0.05$). Only shoots with one single or one pair of double galls were used in this comparison.

Figure 6. 3. 2

Frequency of shoot sharing with other *Pemphigus* galls

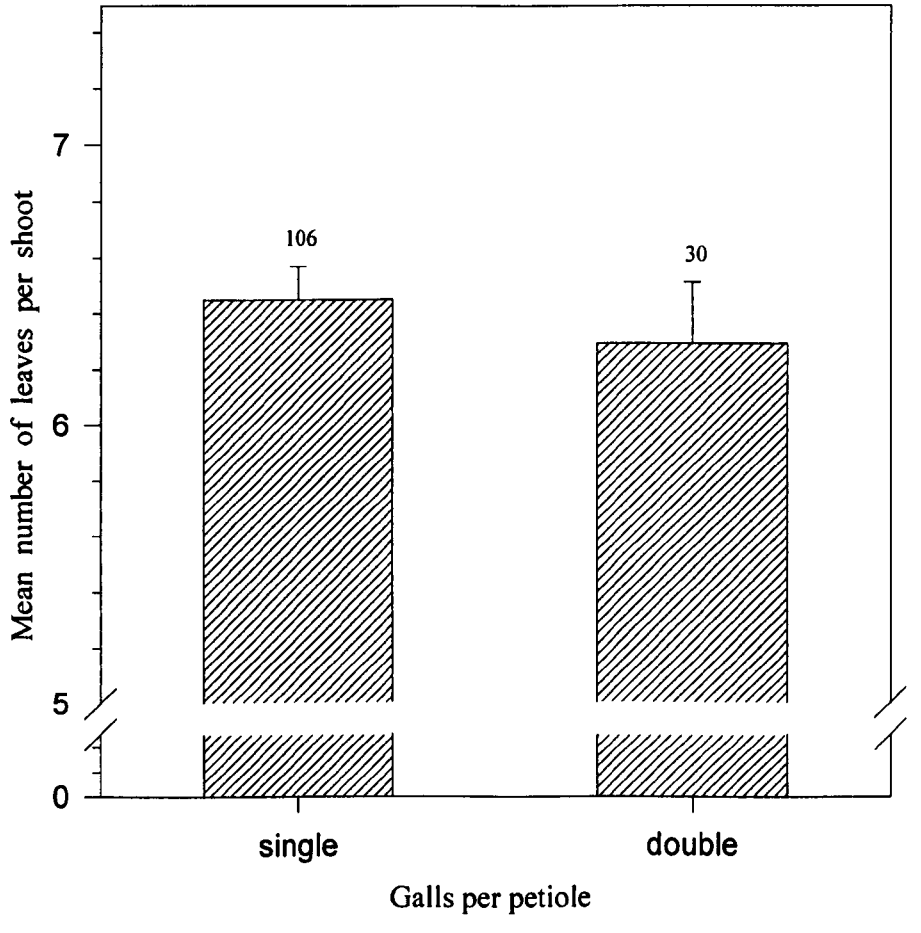


Other *P. spyrothecae* galls on the same shoot as a doubly-galled petiole

This graph illustrates the frequency of doubly-galled petioles occuring on their own or in competition with other *Pemphigus spyrothecae* galls. Double galls are most frequently found with one other single gall on the shoot.

Figure 6. 3. 3

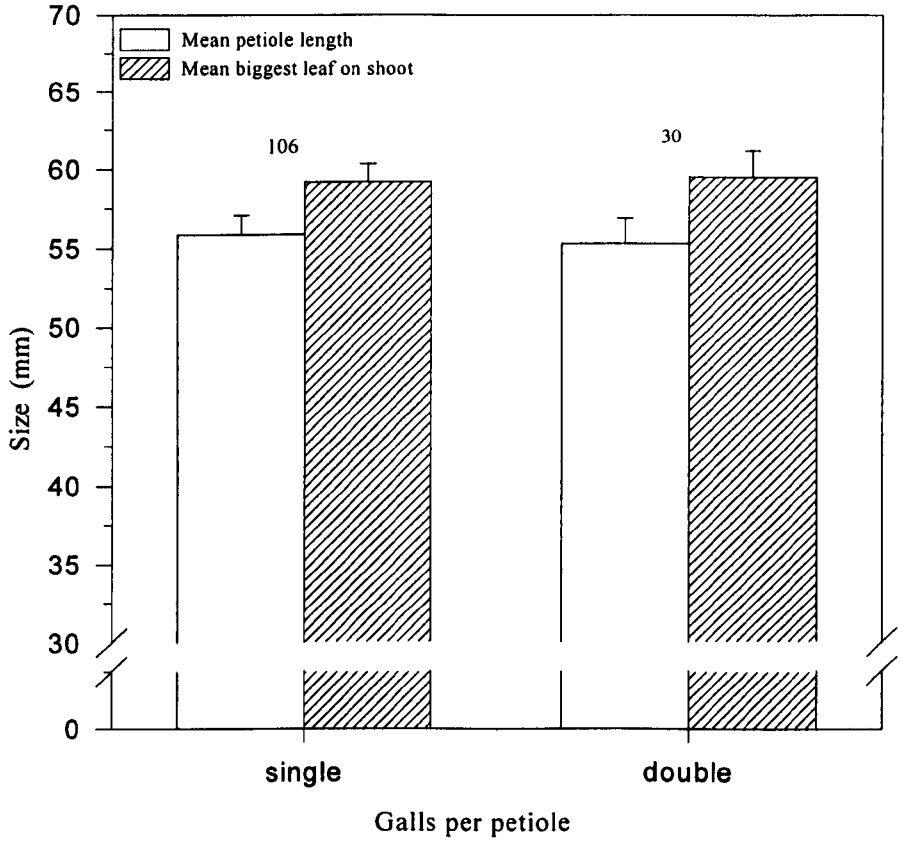
Comparison of poplar shoot size occupied by single and double *Pemphigus* galls



Single and double galls occupied shoots with a similar number of leaves (ANOVA: $F_{1,135} = 0.40$, n.s.). Only shoots with one single or one pair of double galls were used in this comparison.

Figure 6. 3. 4

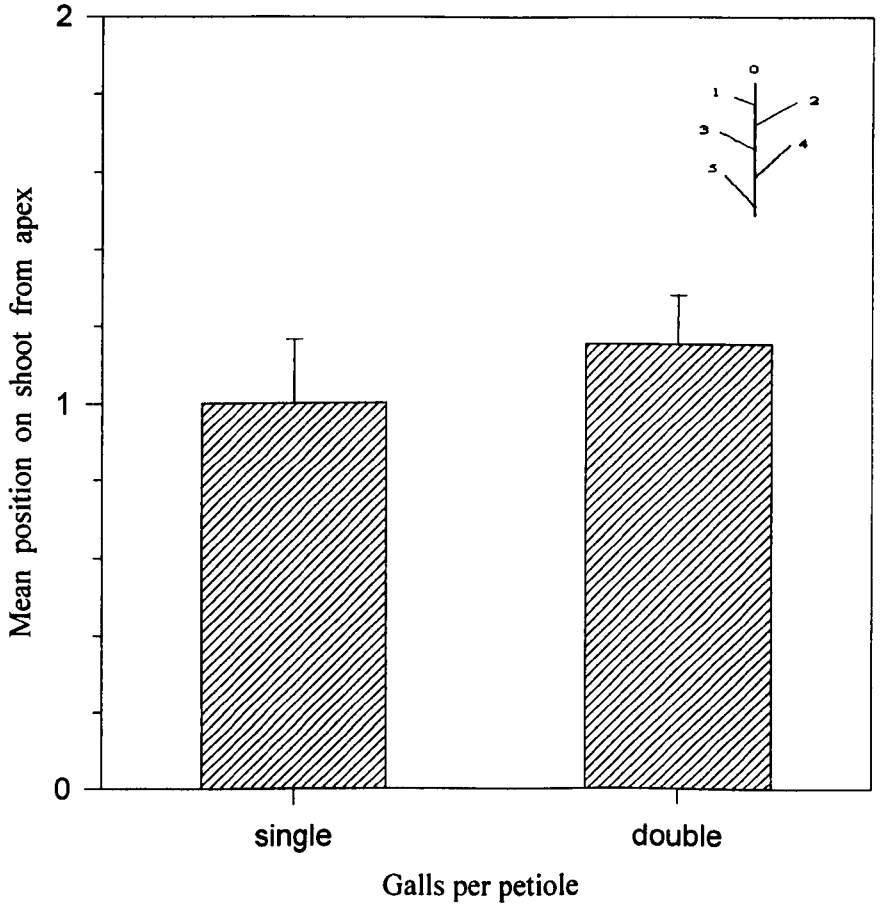
Comparison of shoot and petiole size occupied by single and double *Pemphigus* galls



Single and double *P. spyrothecae* galls occupied poplar petioles of similar size ($F_{1,135}$ = 0.06, n.s.). There were no significant differences between shoot sizes whether occupied by single or double galls ($F_{1,135}$ = 1.77, n.s.). Only shoots with one single or one pair of double galls were used in this comparison. Length of biggest leaf on the shoot is used as an indicator of shoot size.

Figure 6. 3. 5

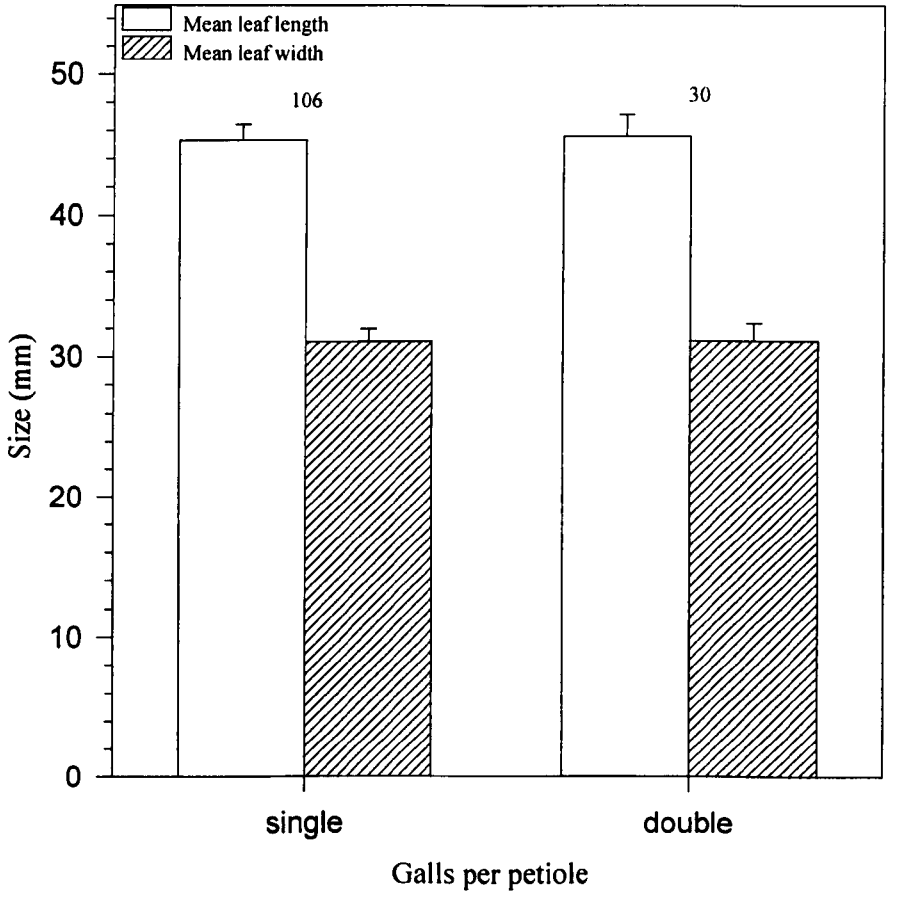
Position of single and double galls on the same poplar shoot



Position of *P. spyrothecae* galls on the same *Populus* shoot. Only shoots with one single plus one pair of double galls per petiole were used in this comparison. Gall position is measured from the apex, with the top petiole equal 0. The doubly-galled petiole was on 24 occasions further away from the shoot apex than the singly-galled petiole. However, there was no apparent difference between the position of the singly or doubly-galled petiole on the shoot (Mann-Whitney U = 685, n.s., n = 40).

Figure 6. 3. 6

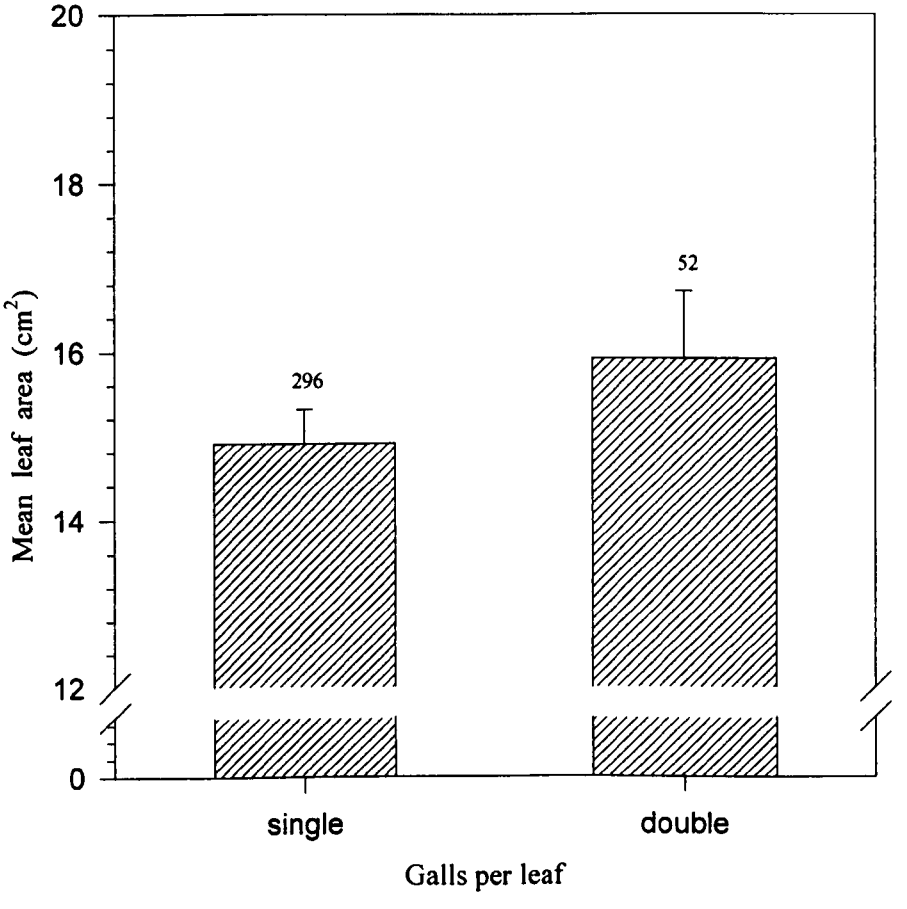
Comparison of leaf sizes occupied by single or double galls



Single and double *P. spyrothecae* galls occupied similar sized poplar leaves (ANOVA: leaf width $F_{1,135} = 0.02$, n.s.; leaf length $F_{1,135} = 1.48$, n.s.). Only shoots with one single or one pair of double galls were used in this comparison.

Figure 6. 3. 7

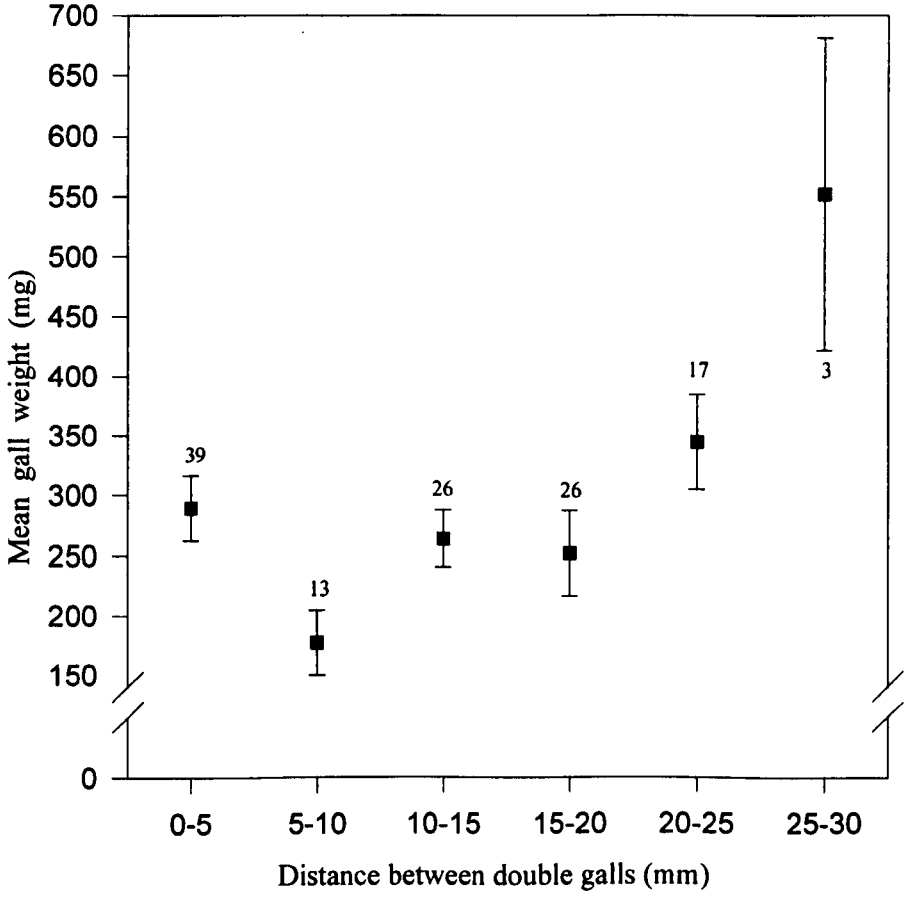
Comparison of leaf area on petioles occupied by single or double galls



Although the average individual poplar leaf area for a doubly-galled petiole was greater than those with only one *Pemphigus* gall, this was not statistically significant (ANOVA: $F_{1,347} = 1.08$, n.s.).

Figure 6. 3. 8

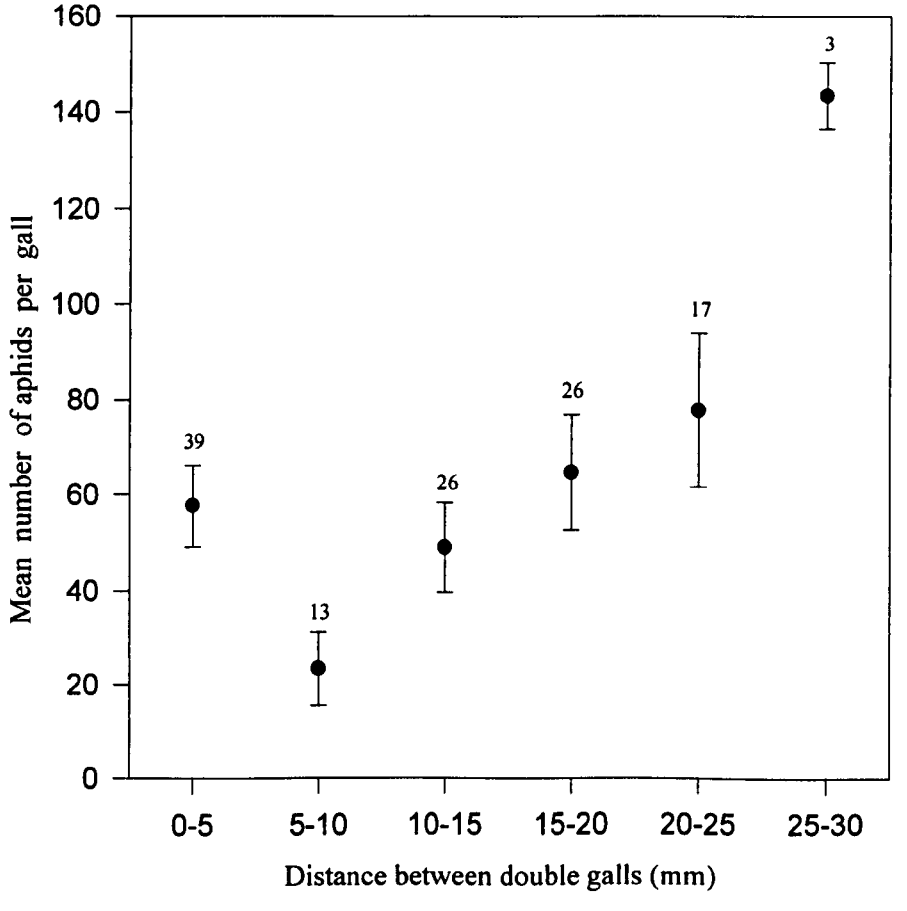
The effect of distance between double galls on a petiole on gall weights



Graph of mean gall weights of *Pemphigus* aphids at different distances on the doubly-galled petioles of *Populus* x *canadensis*. There were significant effects of distance categories between galls on the petiole and gall weights (ANOVA: $F_{5,111} = 3.36$, $p < 0.01$).

Figure 6. 3. 9

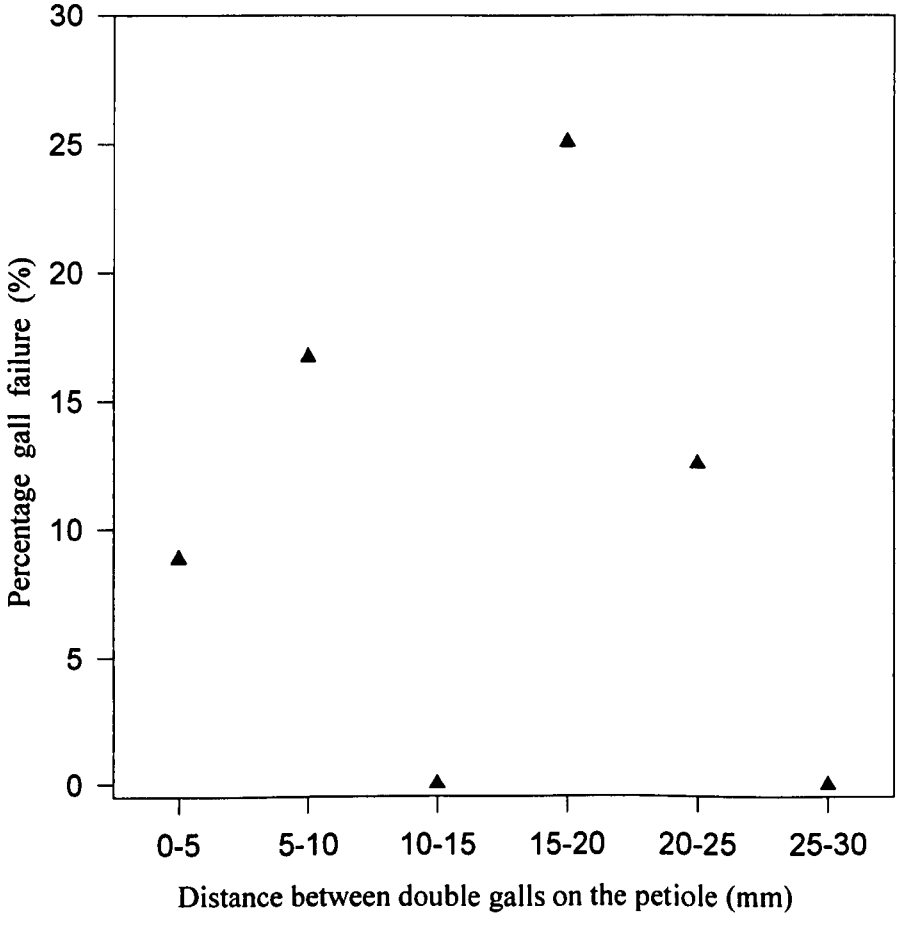
The effect of distance between double galls on a petiole on reproductive success



There were significant effects of distance categories between double *Pemphigus* galls on poplar petioles on reproductive outcome (ANOVA: $F_{5,111} = 2.85$, $p < 0.05$).

Figure 6. 3.10

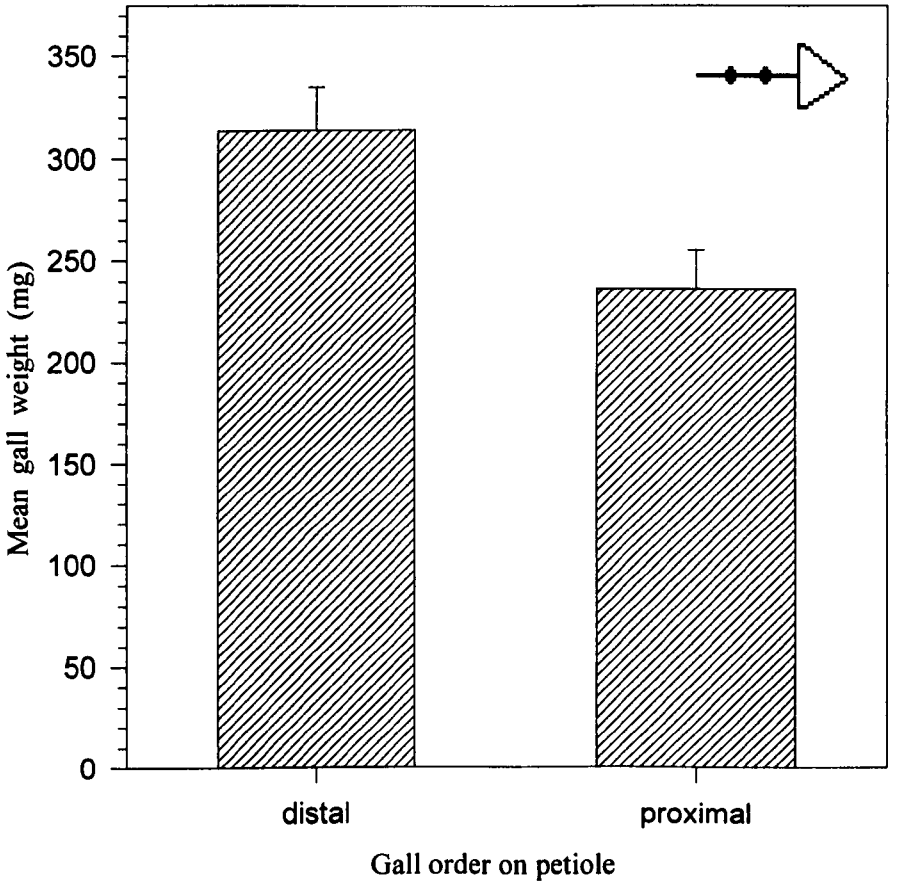
The effect of distance between double galls on a petiole on gall failure



There were no significant effects of distance categories on the probability of *Pemphigus* gall failure of double galls on poplar petioles ($\chi^2_5 = 8.18$, n.s.). Only 16 of the 112 double galls were failures.

Figure 6. 3. 11

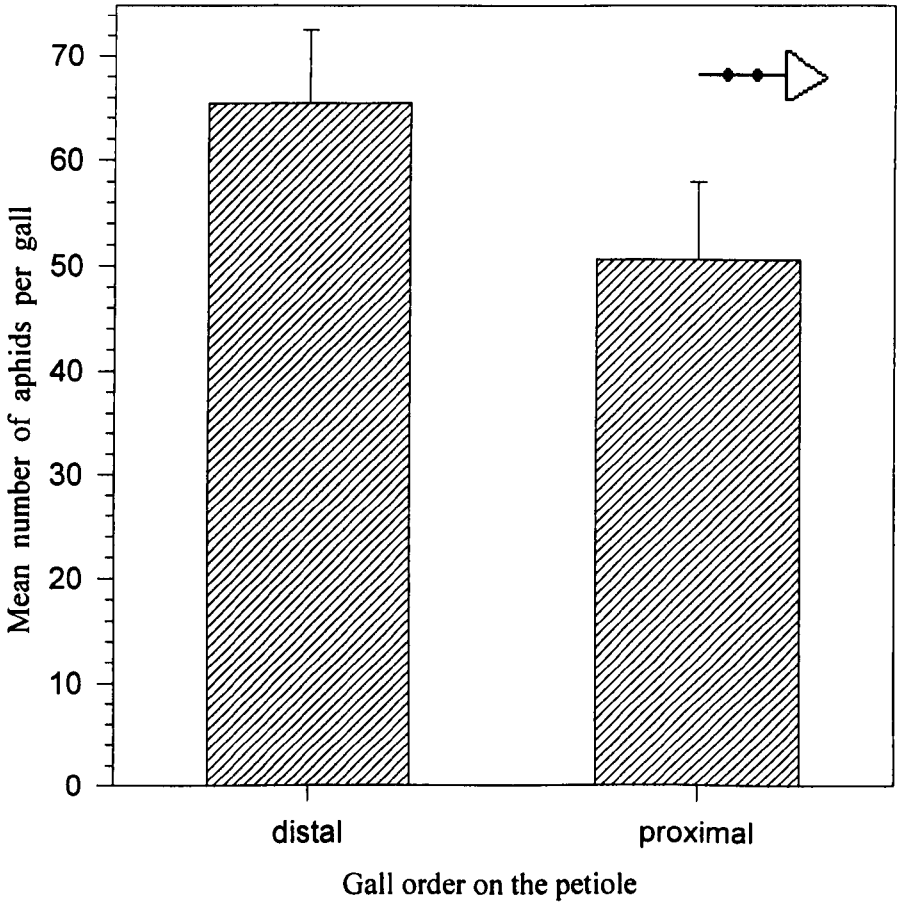
The effect of gall order on gall weight



Gall order had a significant effect on *Pemphigus* gall weight (Paired t-test = 3.53, $p < 0.001$, n = 55). Distal galls were, on average, heavier than proximal (close to the leaf) galls on doubly-galled poplar petioles.

Figure 6. 3. 12

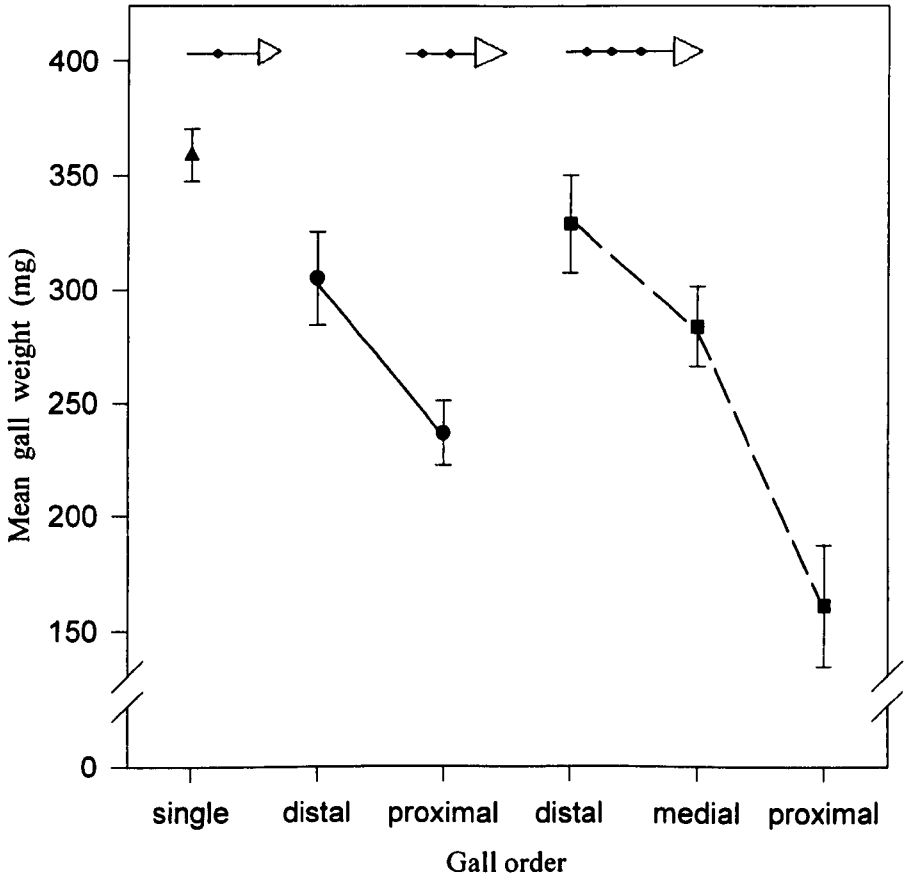
The effect of gall order on reproductive success



Although the average reproductive outcome was larger on *Pemphigus* galls furthest from the poplar leaf (distal), this was not statistically significant (Paired t-test = 1.55, n.s., n = 55).

Figure 6. 3. 13

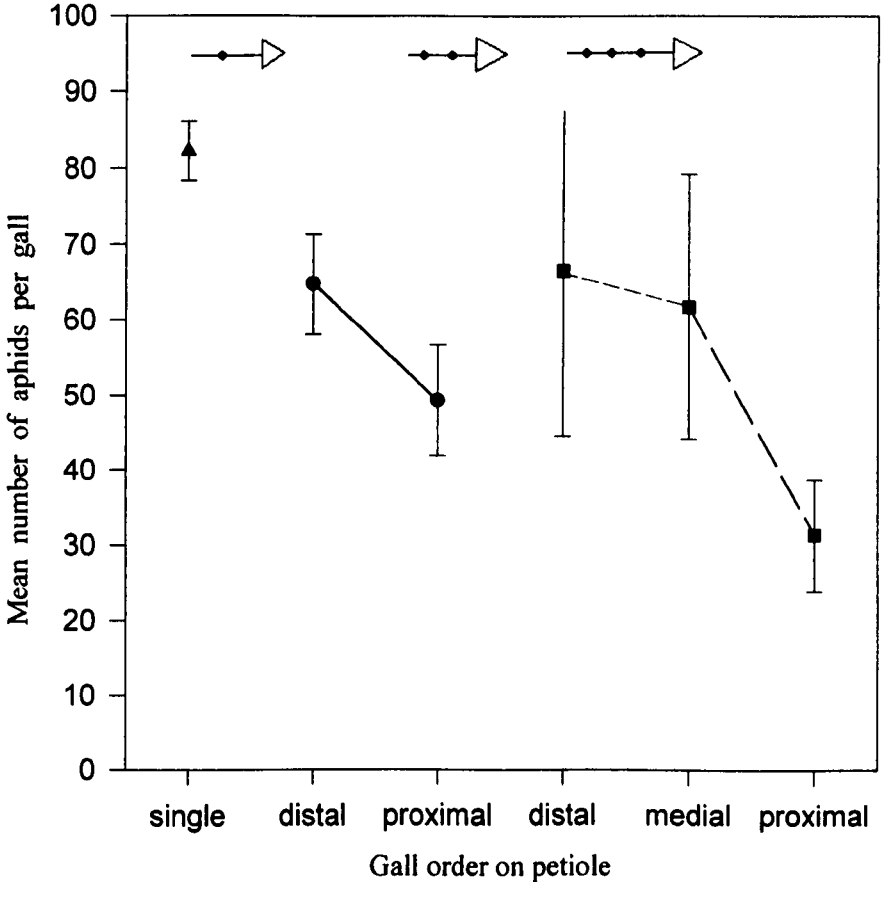
Comparison between single and multiple galls and gall weight



Relationship between *Pemphigus* gall order on poplar petioles and gall weight. To eliminate variation due to leaf size, variables were standardised. This graph includes 296 single, 52 pairs of double and 4 sets of triple galls. Singly-galled petioles had significantly heavier galls than doubly-galled petioles (ANOVA: $F_{1,399} = 17.5$, $p << 0.001$).

Figure 6. 3. 14

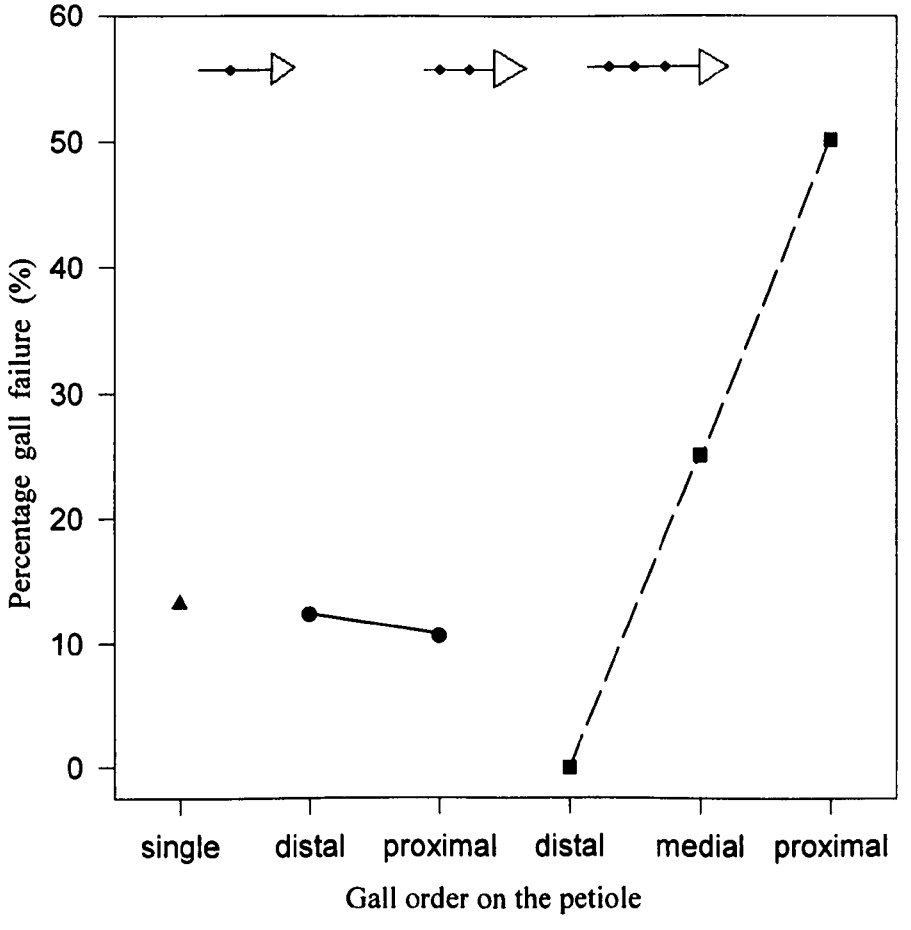
Comparison between single and multiple galls and reproductive success



Relationship between *Pemphigus* gall order on the poplar petiole and reproductive success. To eliminate variation due to leaf size, variables were standardised. This graph includes 296 single, 52 pairs of double and 4 sets of triple galls. Singly-galled petioles carried galls with significantly more aphids than doubly-galled petioles (ANOVA: $F_{1,399} = 12.8$, $p < 0.001$).

Figure 6. 3. 15

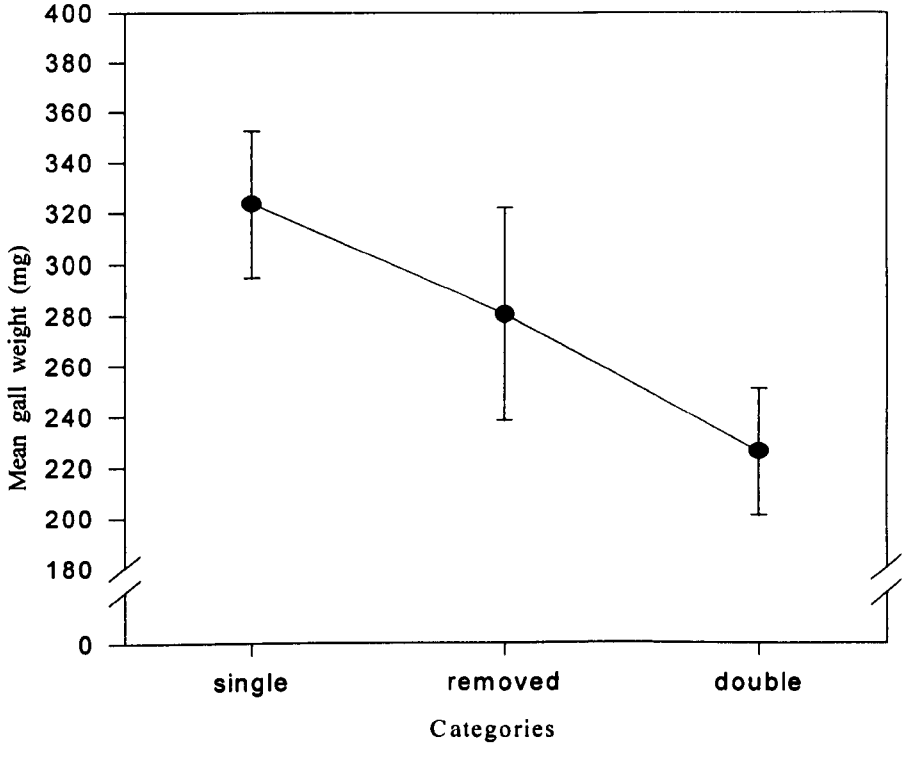
Comparison between single and multiple galls and gall failure



Relationship between *Pemphigus* gall order on the poplar petiole and percentage gall failure. To eliminate variation due to leaf size, variables were standardised. This graph includes 296 single, 52 pairs of double and 4 sets of triple galls. Single and double galls did not differ in percentage gall failure ($\chi^2_2 = 0.25$, n.s.).

Figure 6. 3. 16

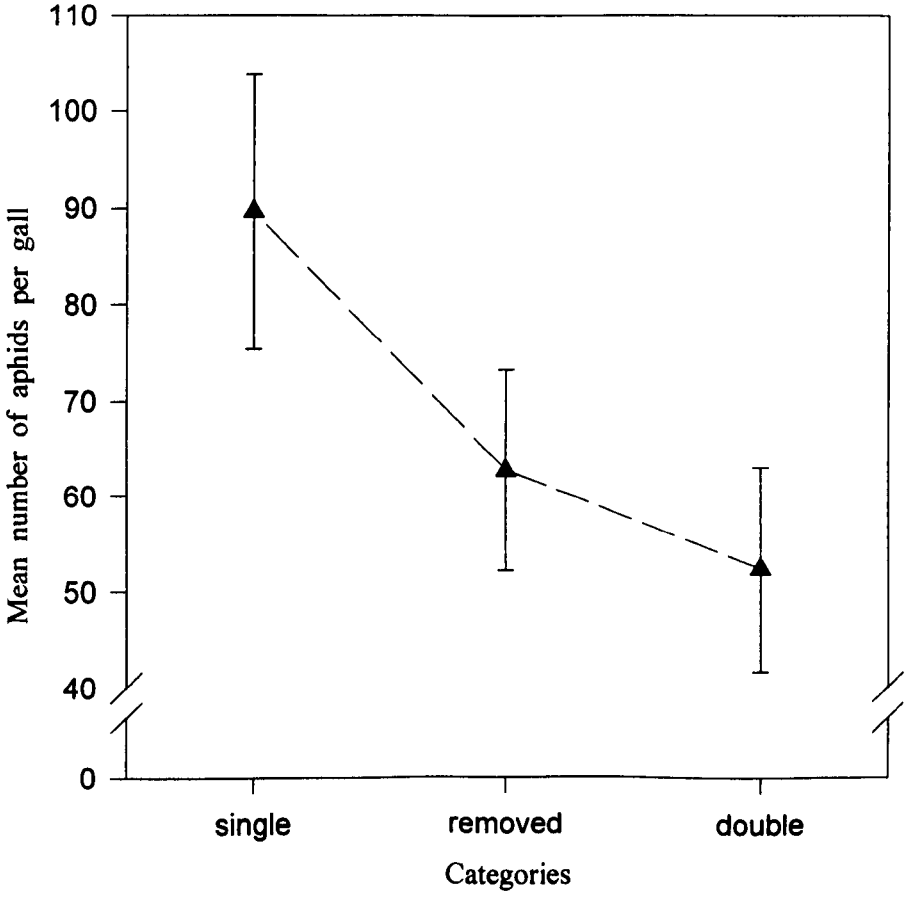
The effect of reproductive release on gall weight



Three *Pemphigus* gall categories were compared: single, double with its proximal (closest to the leaf) partner removed early in the season and double with its proximal gall intact. Although as expected, the average gall weight reduced across categories, this was not significant (repeated measures ANOVA: $F_{2,56} = 2.97$, n.s., n = 19). Galls were matched for leaf and shoot size, and for location on the shoot. A post test comparison between the removed and the double categories was not significant (Bonferroni t = 1.35, n.s.).

Figure 6. 3. 17

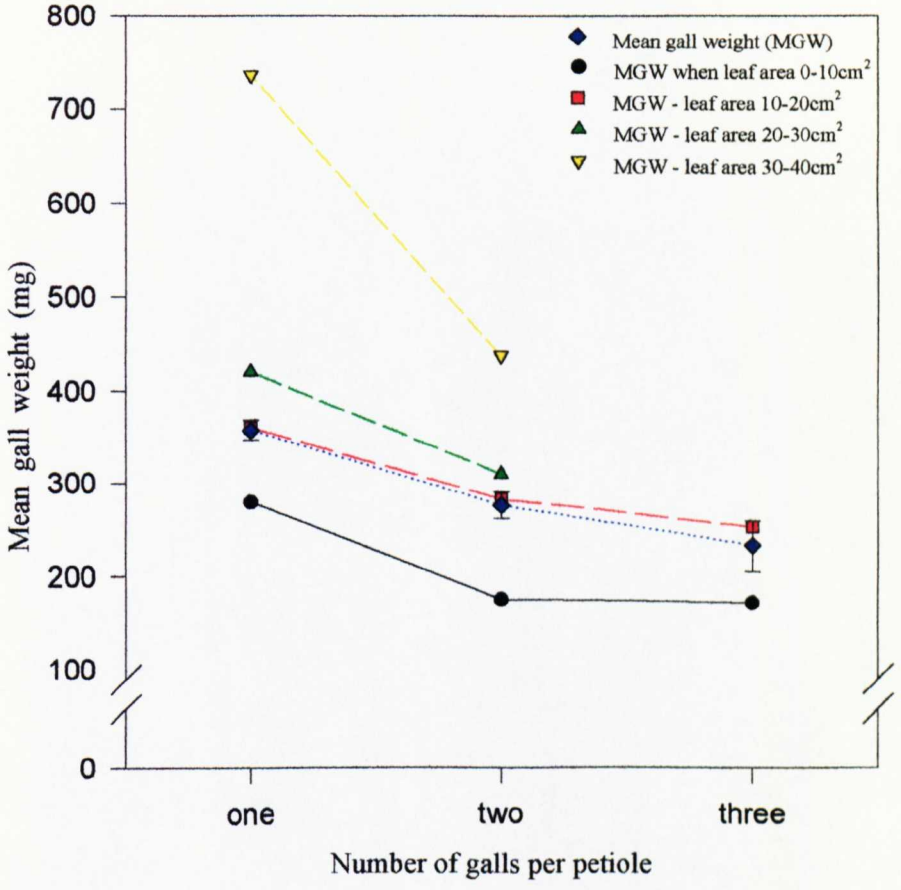
The effect of reproductive release on reproductive success



Three *Pemphigus* gall categories were compared: single, double with its proximal (closest to the leaf) partner removed early in the season and double with its proximal gall intact. The mean number of offspring per gall reduced acros categories, and this was statistically significant (repeated measures ANOVA: $F_{2,56} = 3.34$, $p < 0.05$, n = 19). Galls were matched for leaf and shoot size, and for location on the shoot. However, a post test comparison between the removed and the double categories was not significant (Bonferroni t = 0.69, n.s.).

Figure 6. 3. 18

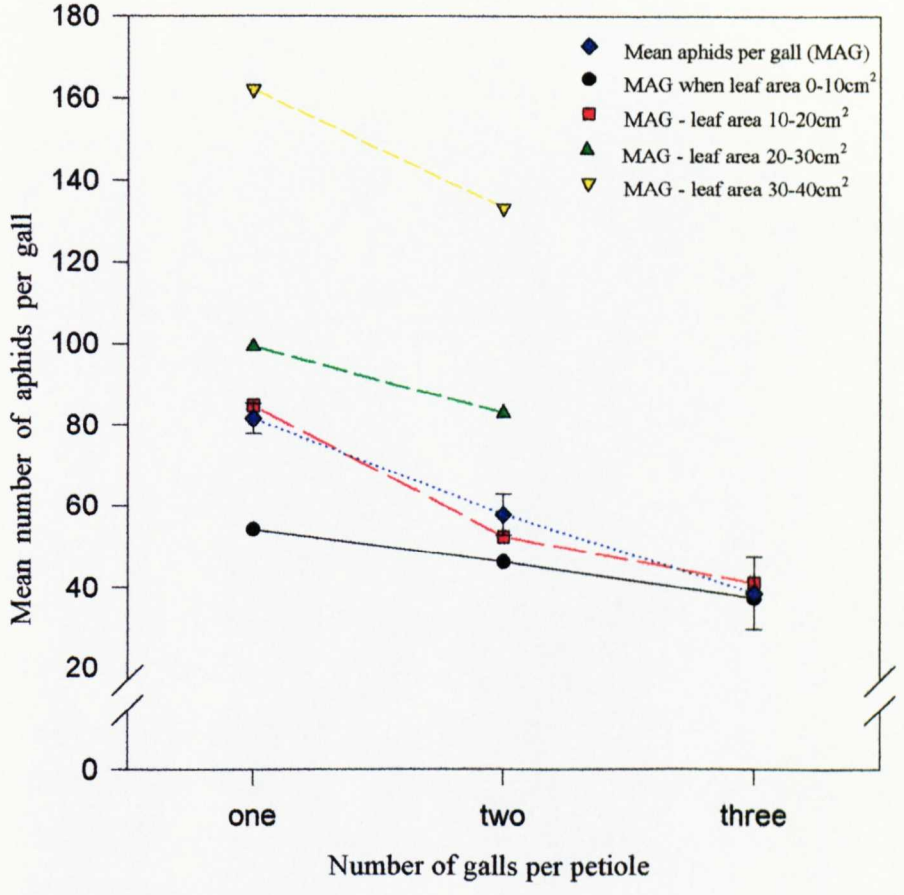
Fitness curve of gall weights on single or shared petioles with different leaf size categories



Fitness curve illustrating *Pemphigus* aphids galling on poplar leaves of varying quality (leaf area) at different competitor densities (one, two or three galls per petiole) together with the average gall weight across all leaf sizes. There was a significant effect of competitor density on average gall weights across all leaf size categories (Kruskal Wallis H = 23.66, $p << 0.001$, n = 459). Only data for one or two galls per petiole were available at leaf areas 20-40 cm^2. An increase in competitor density in a constant environment decreases gall weight.

Figure 6. 3. 19

Fitness curve of reproductive success on single or shared petioles with different leaf size categories



Fitness curve illustrating *Pemphigus* aphids galling on poplar leaves of varying quality (leaf area) at different competitor densities (one, two or three galls per petiole) together with the average number of aphids produced per gall across all leaf sizes. There was a significant effect of competitor density on reproductive success across all leaf size categories (Kruskal Wallis $H = 11.50$, $p < 0.001$, $n = 459$). Only data for one or two galls per petiole were available at leaf areas 20-40 cm^2. An increase in competitor density in a constant environment decreases reproductive outcome.

Chapter 7

DO PREDATORS AFFECT *PEMPHIGUS SPYROTHECAE* GALL DISTRIBUTION AND MORTALITY?

7.1 Introduction

The relationships among predator/prey spatial distributions are complex and have been the subject of many studies (e.g. Morrison, 1986; Waage, 1983). Density-dependent searching behaviour by the predator may in some instances lead to spatial density-dependence of prey mortality in the field. The gall environment may protect the insect from some potential predators, for instance the galling habit reduces the number of parasitoid species attacking nematine sawfly gallers (Price & Pschorn-Walcher, 1988), but, equally, the concentration of aphid biomass in the gall could attract natural enemies.

Tscharntke (1992) found that certain host plant characteristics affected the distribution of midge galls (*Giraudiella inclusa*) on *Phragmites*, influencing the subsequent pattern of parasitism and bird predation. Redfern & Cameron (1994) noted that gall (*Taxomyia taxi*) position on yew trees does not affect the risk of parasitism, but the size of galls has a significant effect on the risk of attack by parasitoids. As is usual with insect herbivores (Strong *et al*, 1984) it is unlikely that *Pemphigus spyrothecae* population growth is often restricted by lack of food, but parasitoids and predators are both a likely source of population regulation.

Although the gall provides protection from the environment and natural predators, galling aphids can suffer a serious reduction in reproductive outcome from specialist enemies (Foster & Northcott, 1994); e.g. Dunn (1966) reported a 90-100% attack rate by the heteropteran bug *Anthocoris* in the galls of *Pemphigus bursarius*. Once inside the gall, a predator can effectively wipe out the entire colony. However, evidence is now mounting that aphids use soldiers to defend the gall from this onslaught. Soldiers of *Pseudoregma*, *Colophina* and *Pemphigus* generally ward off attacks by arthropod

predators, and *Astegopteryx* soldiers may possibly defend the gall from attacks by mammals such as squirrels and Japanese monkeys, or by birds (Aoki,1979). Occasional bird predation on *Pemphigus* galls is also mentioned in Grigarick and Lange (1962). By using artificial galls, Foster (1990) revealed that the soldier caste can kill first-instar ladybirds (*Adalia bipunctata*), third-instar syrphid larvae (*Metasyrphus corollae*) and third-instar *Anthocoris nemoralis* and *Anthocoris minki*. The soldiers, although suffering heavy mortality, were effective at both preventing the entry of the predator, and killing those that did penetrate the gall (Foster, 1990). Foster & Northcott (1994) noted that all soldier-producing aphid species also are gall formers. However, living in a gall is not a prerequisite for a soldier caste, as soldier aphids on secondary hosts are not in galls, and many galling aphid species do not have soldiers. It is possible that soldiers are only produced in those species where the gall is long-lasting and open for a long period, such as in *Pemphigus spyrothecae*. It may simply not be cost efficient to invest in soldiers in short-lived colonies. For a full review on the evolution of soldiers in aphids, see Itô (1989) and Stern & Foster (1996).

Predator behaviour is of great interest, but from a population dynamic point of view, the overall effect of mortality is also important. Several questions needed addressing: a) do *Pemphigus spyrothecae* stem mothers die prior to gall initiation? If so, do predators interfere with *P. spyrothecae* fundatrices attempting to gall at budburst?; b) do predators use plant cues as a guide to gall location?; c) using gall weight as an indicator of size (heavier galls contain more aphids), are heavier galls at greater risk of predation? and d) how does the aphid's natural enemies affect the reproductive success and maturity of the aphids within the gall?

7. 2 Methods

The life cycle of *Pemphigus spyrothecae* Passerini, 1860 has been described by Dunn (1966) and Foster (1990). This species is not host alternating, but spends its whole life cycle on its primary host, the black poplar *Populus nigra* and its hybrids and varieties. Eggs laid the previous year in the bark on branches or on the trunk of the host tree, hatch in spring and produce parthenogenetic stem mothers (first generation). The wingless stem mothers are very mobile, and migrate out to the newly emerged shoots, where they select a petiole for gall formation. By midsummer up to several hundred offspring are parthenogenetically produced. The first instars grow into wingless adults (virginoparae, second generation), and these in turn produce both thick-legged and normal-legged first instars. The normal-legged instars grow into winged sexuparae (third generation), that, once the galls have opened in August, migrate to the trunk, give birth to sexuals (fourth generation), which mate and produce overwintering eggs in the bark of the tree.

The hybrid black poplar (*Populus* x *canadensis*) trees used in this study are located at Attenborough Gravel Pits, Nottingham, England, a Site of Special Scientific Interest (SSSI), jointly managed by Nottinghamshire Wildlife Trust and owners Butterley Aggregates Ltd. Mature trees were used, as juvenile trees are resistant to galling and are avoided by migrants (Kearsley & Whitham, 1989). The trees grow close together along a stream near a disused car park, and hence are all subjected to similar climatic and environmental conditions. The trees were planted approximately 30 years ago.

P. spyrothecae stem mothers emerge in early spring from crevices in the bark on the trunks of hybrid black poplars. The fundatrix migrates to newly opened shoots where she begins to form a spiral gall on a suitable petiole. On the first day of emergence (18th April) I choose 15 poplar shoots at random from a tree, and applied a barrier of sticky paste (made of Lanolin and water) around the base of each shoot. This prevented stem mothers already on the shoot from escaping and new aphids from entering. I recorded the number of live and dead stem mothers on each shoot and repeated these recordings the next two days. When *P. spyrothecae* stem mothers hatched the following spring on the trees at Attenborough Nature Reserve, I choose

one branch with 112 shoots on, and examined these daily for 30 days for evidence of predator activity. On the 5th of May I censused 1000 galls at random from 8 poplars and recorded other insects present. Towards the end of August, prior to alate dispersal, I collected and dissected 500 *Pemphigus spyrothecae* galls and again noted any evidence of natural enemies.

In spring when gall formation was completed, ten single galls were caged on each of eight *Populus* x *canadensis* trees. By wrapping a piece of sticky tape around the aphid gall this effectively enclosed the galls, allowing no predators to enter. To control for negative effects of density, when more than one stem mother colonises the same petiole, only single galls were included in this experiment. Each caged gall was matched by a control gall in terms of leaf size, gall size, gall position on the shoot and general position on the tree. For convenience I selected branches with galls all within arms reach. The caged galls, together with the matching controls, were collected in August prior to alate dispersal. Since *P. spyrothecae* galls are initiated within the same time period in spring the data collected represent a cohort. Together with the 500 galls collected above, the galled leaves were measured for greatest length and width in mm, and leaf tissue area was calculated by placing the leaf under a 1 cm^2 transparent grid, and summing the number of 1 cm^2 units within the leaf outlines. Petiole lengths were measured in mm and the number of galls per petiole recorded.

Gall wet weight was recorded in mg on a Mettler PJ 400 weighing machine. Counting the number of progeny per gall indicates the individual fitness of each stem mother. The galls were opened and the occupants gently transferred with a small paintbrush into a petri-dish containing 70% methcol, and the number of aphids counted under a Wild M3 dissecting microscope. Any non-aphid inhabitants or predators were noted and retained for identification. The design of this experiment is that of matched pairs, and therefore I used either a paired 't'-test (parametric) or Wilcoxon matched pairs rank test (non-parametric). This automatically allows for among-tree differences since the galls were matched. Additionally I was interested in whether there were among-tree effects: I therefore subjected the differences ('caged' minus 'control') between pairs to 1-way ANOVA, with 'trees' as a factor with 8 levels.

7. 3 Results

Following *Pemphigus spyrothecae* emergence (mid-April in the study year), stem mothers face a vulnerable time locating a suitable galling site on the petioles of *Populus* x *canadensis* shoots. Fig. 7.3.1 illustrates the number of live stem mothers on 15 poplar shoots chosen at random. Just a couple of days later, many shoots have lost more than half of the original number of colonisers. Stem mother survival rate ranged between 33-100% with an average of just under 60% during these first three days (Fig. 7.3.2).

Aphid colonies are at the mercy of many natural predators such as ladybirds and their larvae and the larvae of hoverflies and lacewings. During the month of April, I examined 112 shoots for the presence of predators every day. *Chaitophorus leucomelas*, a green aphid, was first detected on the poplar buds in early April and is commonly found in association with *Pemphigus spyrothecae* (Foster, 1990). I only noted eight other insects namely; three leafhoppers (probably *Idiocerus vitreus*), one froghopper, one small parasitic wasp, one weevil, one small caterpillar, and one black beetle. On 5th of May I censused 1000 galled shoots and found 16 leafhoppers, five froghoppers, two mayflies, two ladybirds, six midges, one caterpillar, and colonies of the aphid *Pterocomma populeum*, a densely haired and cryptically coloured aphid living on the bark of willows and poplars. Although, I did not dissect the censused galls, they were all subjected to a close scrutiny for predators.

Towards the end of August I collected and dissected 500 *P. spyrothecae* galls and on only a few occasions found any evidence of predator occupation, which initially could indicate that predator numbers were low in the study year. However, on many of the caged galls (experimental results given below), the wind and rain had dissolved small patches of glue, allowing visiting insects to enter the perimeter of the cage. This provided an excellent record of which species and how many individuals were visiting the galls. In addition to *Anthocoris minki*, I noted species of earwigs, leaf hoppers, ladybirds, and other Heteroptera such as *Miris* sp. and the Black-knee capsid, *Blepharidopterus angulatus*. Two species of ants were seen, *Myrmica* sp. and *Lasius niger*. Fig. 7.3.3 illustrates how many visitors were collected from the 78 adhesive

sticky tapes surrounding the caged galls. By far the most abundant natural enemy is *Anthocoris minki*. A further 20 *Anthocoris minki* were found on, in or near the control galls. This indicates that the galls are not being attacked on mass, but rather visited regularly throughout the season. In order to ascertain whether the main predator *Anthocoris minki* was predominantly found on any particular tree, I examined among-tree variation in numbers. However, I found that all eight trees were equally foraged by *Anthocoris* (Kruskal Wallis H = 0.614, n.s.).

To examine whether predators of *P. spyrothecae* aphids used plant properties as a cue to locate aphid populations, I compared gall sites where predators were either present or absent. I found no significant effects of either the length or the width of galled leaves (Fig. 7.3.4) nor the leaf tissue surface area (Fig. 7.3.5). Leaf damage did not affect predator foraging choice (Fig. 7.3.6). However, predators were more likely to be found in or near *Pemphigus spyrothecae* galls on smaller sized petioles (Fig.7.3.7) with fewer galls (Fig. 7.3.8), and although predators approached, on average, heavier sized galls, this was not statistically significant (Fig. 7.3.9).

To examine how a *Pemphigus spyrothecae* gall is affected by predation, I recorded three different indicators of aphid fitness, namely: gall weight, number of aphids per gall and number of alates per gall. Following gall completion, one half of the experimental galls were enclosed in cages to keep out intruders, and the other half, matching in position and leaf area size, were left untouched allowing potential predators full access to the inhabitants of the gall. As two caged galls were not recovered in the autumn, 132 caged and 132 control galls were dissected. Gall weights were nearly identical (Fig. 7.3.10). Although the caged galls had, on average, more nymphs and adults per gall, this was not statistically significant (Figs. 7.3.11 and 7.3.12). To ascertain whether there were among-tree effects, i.e. did some trees have a greater difference between the pairs of caged and control galls and aphid populations, a 1-way ANOVA suggested that all eight trees had similar mean differences (differences in gall weight between pairs of caged and control galls $F_{7,123}$ =1.377, n.s; differences in aphid numbers $F_{7,123}$ = 0.78, n.s.; differences in winged progeny $F_{7,123}$ = 1.033, n.s.).

7. 4 Discussion

In a previous study I noted that different climatic variables, such as wind, rain and humidity, are highly correlated, and therefore it is difficult to determine a single factor as being the major contributor to *Pemphigus spyrothecae* aphid mortality during colonisation. Wind batters poplar leaves and aphids may become dislodged and fall to the ground, or washed off the leaves and petioles by rain. As Fig. 7.3.2 suggests, *Pemphigus spyrothecae* fundatrices face a high mortality rate in the first few days, but once enclosed within the gall, stem mothers are protected from the elements. However, during this stage of *Pemphigus spyrothecae*'s lifecycle the aphid is sedentary and has no means of escape from its enemies. The gall environment may protect the aphids from potential predators, but the concentration of aphid biomass may also be attractive to foraging enemies.

Fig. 7.3.3 illustrates what insect species were drawn towards *P. spyrothecae* galls, but does not include insects that may have approached the encased galls during the season, but were not captured by the tape. Phloem-feeding Homoptera are often seen tended by ants that harvest their energy rich honeydew. In return ants can provide a range of benefits to homopterans including protection from predators and parasitoids (Way, 1963), by either biting or exuding drops of formic acid. Predation on homopteran populations can be severe, especially at early developmental stages, so ant protection can greatly decrease the number of fatalities (Crushman & Whitham, 1989). Interestingly, on a few occasions both the black garden ant (*Lasius*) and adult ladybirds (*Adalia bipunctata* and *Coccinella 7-punctata*) were found on the same piece of sticky tape. Other visitors, such as earwigs, are essentially nocturnal and forage on both plant and animal matter, and Miridae e.g. the Black-kneed capsid, are also partly predatory. Anthocorid bugs are quite common in several species of galls, such as *Smynthurodes betae* and *Aploneura lentisci*. In this study *Anthocoris minki* was by far the most abundant predator of *Pemphigus spyrothecae,* but *Anthocoris nemoralis* is also known to forage in *P. spyrothecae* galls, and *Anthocoris antevolens* has been recorded preying on *Pemphigus* aphids in Canada (Harper, 1963). Other Coccinellid beetles to prey on galling aphids include *Scymnus cervicalis* on grape

phylloxera galls (Wheeler & Jubb, 1979) and *Scymnus* sp. on *Pemphigus* galls (Harper, 1963).

Predators enter galls by boring into them or through an opening. A common predator on *Fordinae* galls in Israel and Iran is a small pyralid moth (*Alophia* sp.). This moth enters galls of several species including *Baizongia pistaciae* and *Smynthurodes betae* (Wool, 1984). Aphid predators also include Diptera, especially *Chamaemyidae* and *Syrphidae*. At least two Chamaemyid flies feed on and pupate in galls of *Forda marginata* and *Aploneura lentisci* infesting up to 80% of galls in any season. Maggots of the syrphid *Meliscaeva auricollis* have also been found in galls of *Aploneura lentisci*. This species usually feeds on free-living aphids, so whether the occurrence in galls is accidental is unknown. Wool (1984) noticed that a large number of *Geoica utricularia* galls on *Pistacia atlantica* were punctured and empty. He suspects that birds were responsible, as observations in Japan indicate that sparrows are able to tear the top part of galls of two species of aphids and feed on the enclosed inhabitants (Sunose, 1980). The birds do not eat gall tissue, so this behaviour may have come about by mistaking the gall for fruit. Occasional bird predation on *Pemphigus* galls is mentioned in Grigarick and Lange (1968). All these predators, however common, seem to have little effect on the aphid population size. Reproduction within the gall is parthenogenetic, so even a few survivors of predation are sufficient to ensure the survival of the 'genotype'.

How predators of *Pemphigus spyrothecae* locate the galls remains unclear. With the exception of shorter petioles being more attractive to predators, Figs. 7.3.4-6 suggest that leaf structure was not important. Whitham (1978) likewise found no differences in predator and predator free galled leaf sizes. Since predators appear to forage irrespective of leaf size, and since large leaves are relatively scarce increasing predator foraging costs, the selective advantage of galling on a large leaf remains. Foraging enemies may also obtain chemical information from the herbivore, from its food source, from organisms associated with the herbivore, or from interactions between these sources. Any product from the herbivore can potentially provide a chemical cue for its attackers, for instance faeces, cuticle, pheromones, honeydew or hemolymph (Vet & Dicke, 1992).

One cornerstone of biological control has been that natural enemies should act in a density-dependent manner. However, many studies (e.g. Murdoch *et al*, 1984; Morrison, 1986; Stiling, 1987) suggest that the normal dynamics of insect predator-prey associations are spatially density-independent. Walton (1988) found the pattern of mortality of *Eurosta* larvae was neither density-dependent nor aggregated independently of gall density, and his results suggest that predator/parasite searching strategies probably do not affect the distribution of *Eurosta solidaginis*. In this study the predators of *Pemphigus spyrothecae* aphids were more likely to be found on petioles with fewer galls (Fig. 7.3.8), suggesting an inverse density-dependent searching strategy. Stiling (1987) reviewed 171 case histories of host abundance and related parasitism rates, and discovered density-dependent mortality in 25% of insect systems with inverse density-dependent mortality in 23%, and independence in the remaining 52% of systems.

Variation in the risk of predation or parasitism with gall size is known in a number of insects (e.g. Weis *et al*,1985; Price & Clancy, 1986; Sitch *et al*, 1988) suggesting that a non-random risk of attack by predators and parasites affects herbivore population dynamics (Hassell & Anderson, 1989). Price & Clancy (1986) and Weis *et al* (1985) both report cases of parasitoid selection against smaller than average galls. Some sawfly (*Euura lasiolepis*) galls are invulnerable to parasitoid (*Pteromalus spp.*) attack because the thickness of the gall walls is greater than the length of *Pteromalus* ovipositors (Price & Clancy, 1986). In this study predators of *Pemphigus spyrothecae* aphids tended to be found, although not statistically significant, at larger galls (which usually contain more aphids). Studies by Confer & Paicos (1985) and Tscharntke (1992) noted that bird predators also favoured large galls or gall clusters.

The mean *Pemphigus spyrothecae* gall weights of the caged and control galls were nearly identical (Fig.7.3.10). Caged and control galls showed no significant difference in the number of progeny in the galls (Fig.7.3.11) or the number of alates that occupied each (Fig.7.3.12), and this was shown to be the case across all eight *Populus* x *canadensis* trees. There are several interpretations of these results: a) predators are ineffectual normally; b) predators are not abundant enough; c) predators are

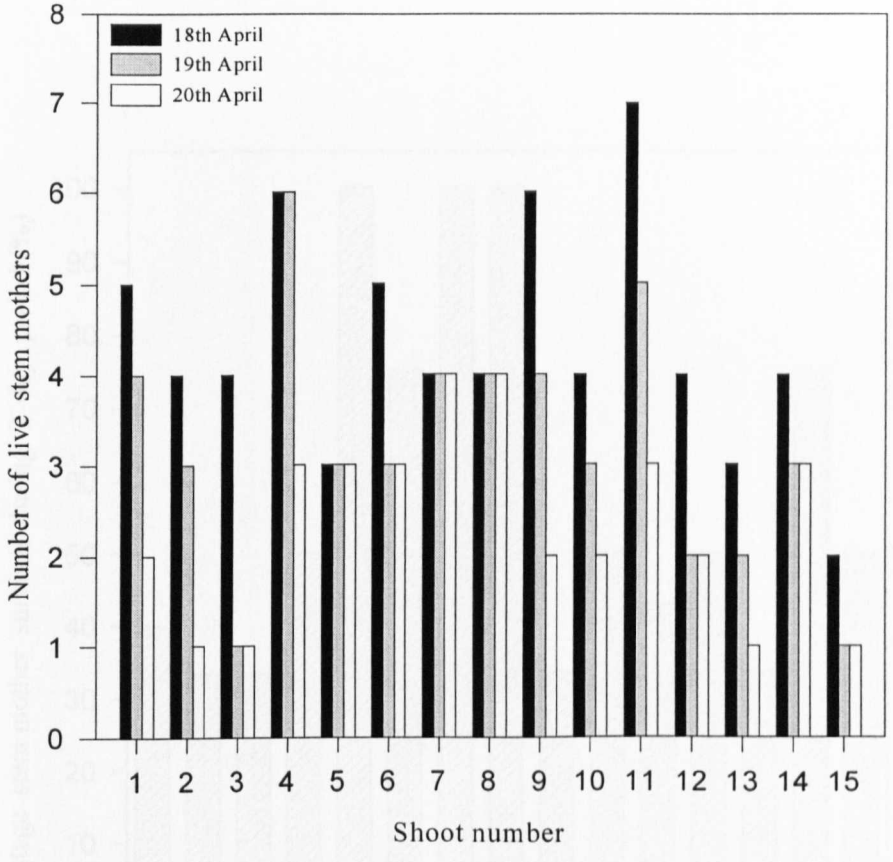
ineffective only in the study year because of low abundance; and d) predators are kept out by soldiers. However, a): aphid predators such as *Anthocoris* bugs are ferocious killers, and Dunn (1966) reported a single *Anthocoris* sp. killing and feeding on the entire *Pemphigus* gall population. b) and c): the caged experiment suggests that many predators were evident in the study year; over 140 *Anthocoris minki* were found stuck onto the adhesive tape used to encase galls. d): Foster (1990) reported that *Pemphigus spyrothecae* soldiers are able to prevent a range of predators from entering the gall and will kill any that do. Soldiers also protect colony members, and if an enemy penetrates the gall, significantly fewer non-soldiers than soldiers die. This is therefore likely to be the most plausible explanation why the caged experiment failed to produce any significant results.

Foster & Rhoden (1998) manipulated the number of *Pemphigus spyrothecae* soldiers in galls in an attempt to ascertain how soldiers reduce the risk of colony mortality. Galls with 50 soldiers and 50 non-soldiers were 10 times less likely to be attacked by predators than galls with 100 non-soldiers. They counted fewer live aphids and significantly more dead aphids in galls without soldiers than in those with soldiers or galls enclosed within a bag earlier in the season. Similarly to the present study, Foster & Rhoden (1998) reported no difference in survival between bagged galls and those protected by soldiers.

Finally, Price & Pschorn-Walcher (1988) reported that the galling habit had a long-term impact on the population dynamics of a nematine sawfly by reducing both the number of attacking parasitoid species and the percentage mortality inflicted. They theorise that natural enemies may have been important as a selective factor in the evolution of galling nematine sawflies. The evolution of a soldier caste offers further protection against predation, and although may be costly in terms of a reduction in the number of fertile progeny in some aphid species, is compensated for by the survival of the colony.

Figure 7. 3. 1

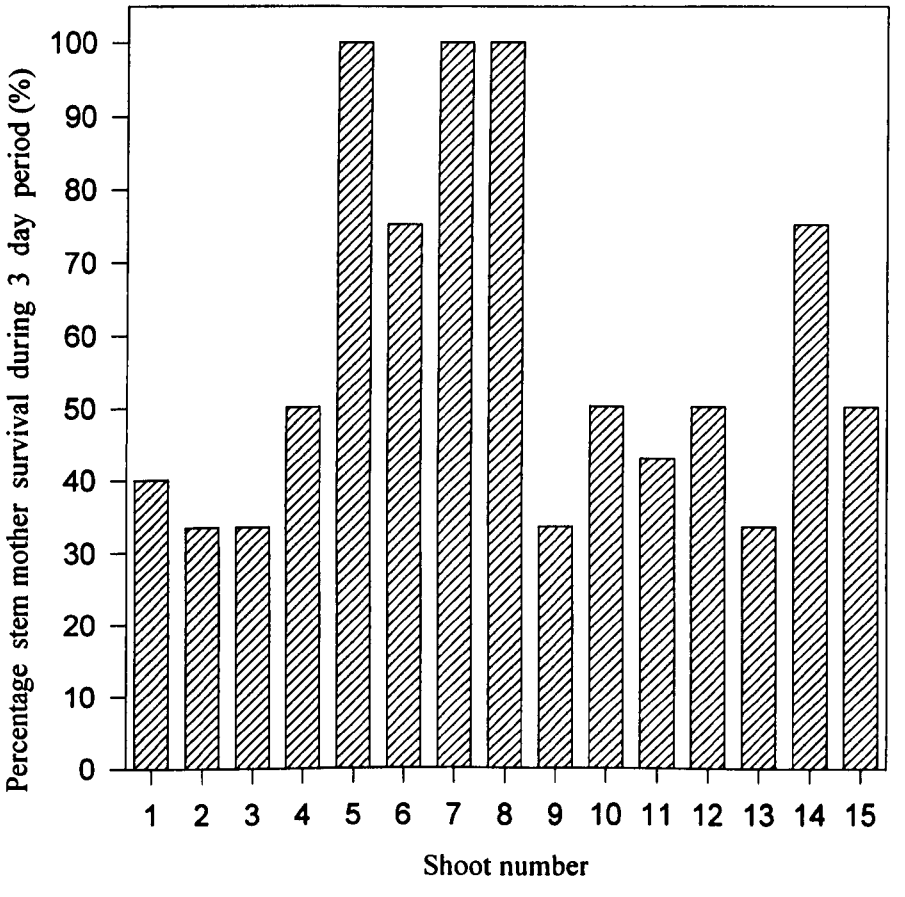
Aphid survival during colonisation on 15 shoots



Pemphigus spyrothecae fundatrices emerge in spring (in the study year April 18th) and migrate to shoots where galls are formed on leaf petioles. In most cases only two days later there was a significant drop in survival, with just three shoots having the original number of colonisers. Migration from the shoot was prevented by using Lanolin mixed with water to form a paste and applied at the base of the shoot as a barrier.

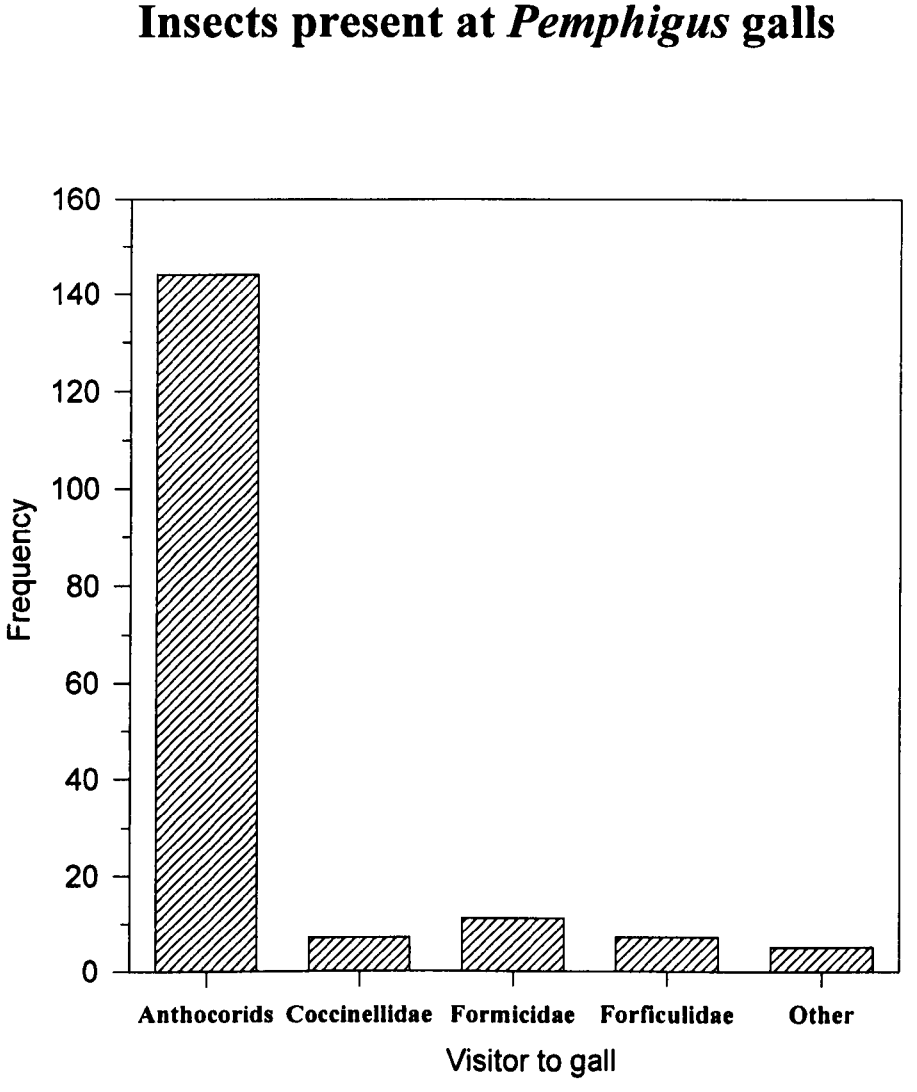
Figure 7. 3. 2

Stem mother survival on 15 shoots 3 days following emergence



P. spyrothecae survival on 15 poplar shoots during the colonisation period. Lanolin was mixed with water to form a paste, which was used as a barrier to prevent aphids from entering or leaving the shoots. An average of 40% of stem mothers died or disappeared from the shoots (presumably fell off) just two days after emergence (April 18th).

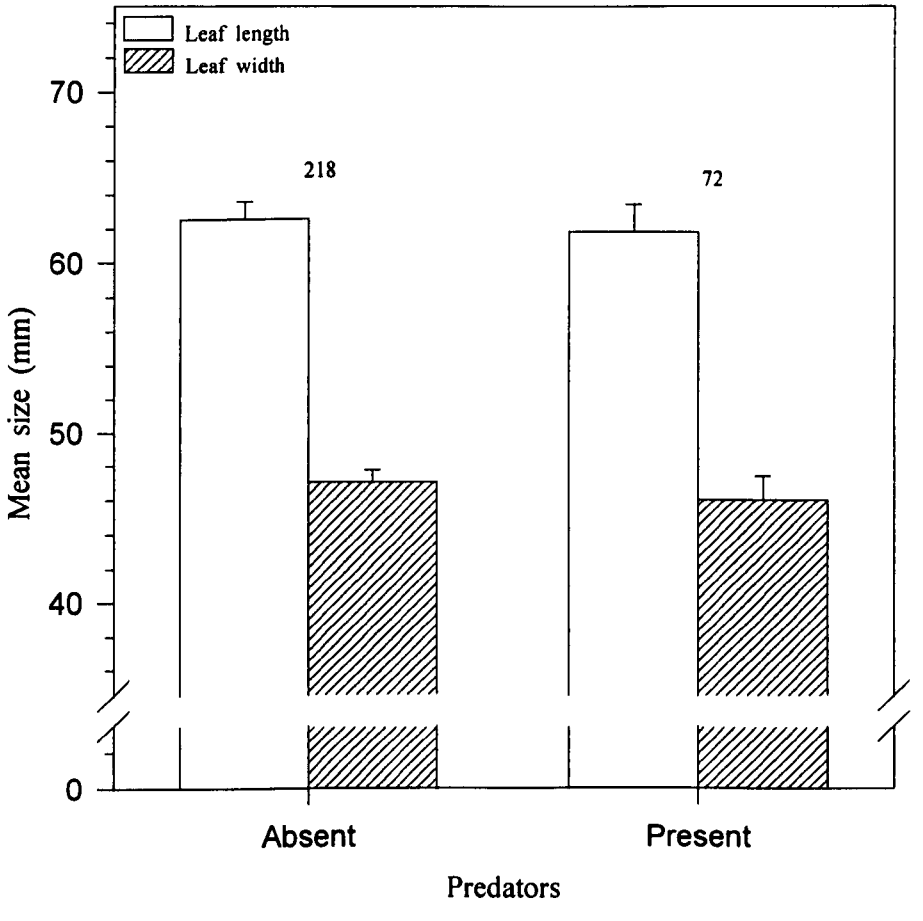
Figure 7. 3. 3



A total of 174 visitors to 78 caged galls were captured on the adhesive of the sticky tape which enclosed the galls from late spring to early autumn. *Anthocoris minki* is the main predator of *Pemphigus spyrothecae*. Within the gall it can devastate the entire colony.

Figure 7. 3. 4

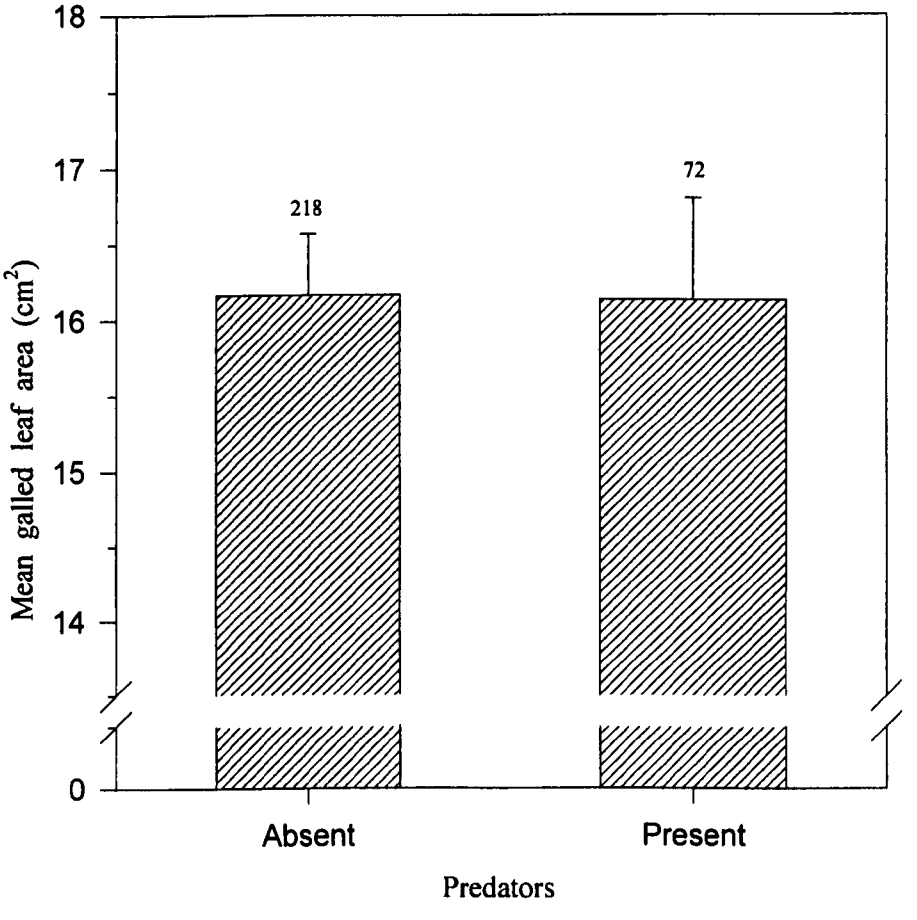
Predators in *Pemphigus spyrothecae* galls on poplar leaves of different sizes



There were no significant effects of *Populus* x *canadensis* leaf length or width on predator choice of foraging (ANOVA $F_{1,289}$ = 0.185, n.s. and $F_{1,289}$ = 0.430, n.s. respectively).

Figure 7. 3. 5

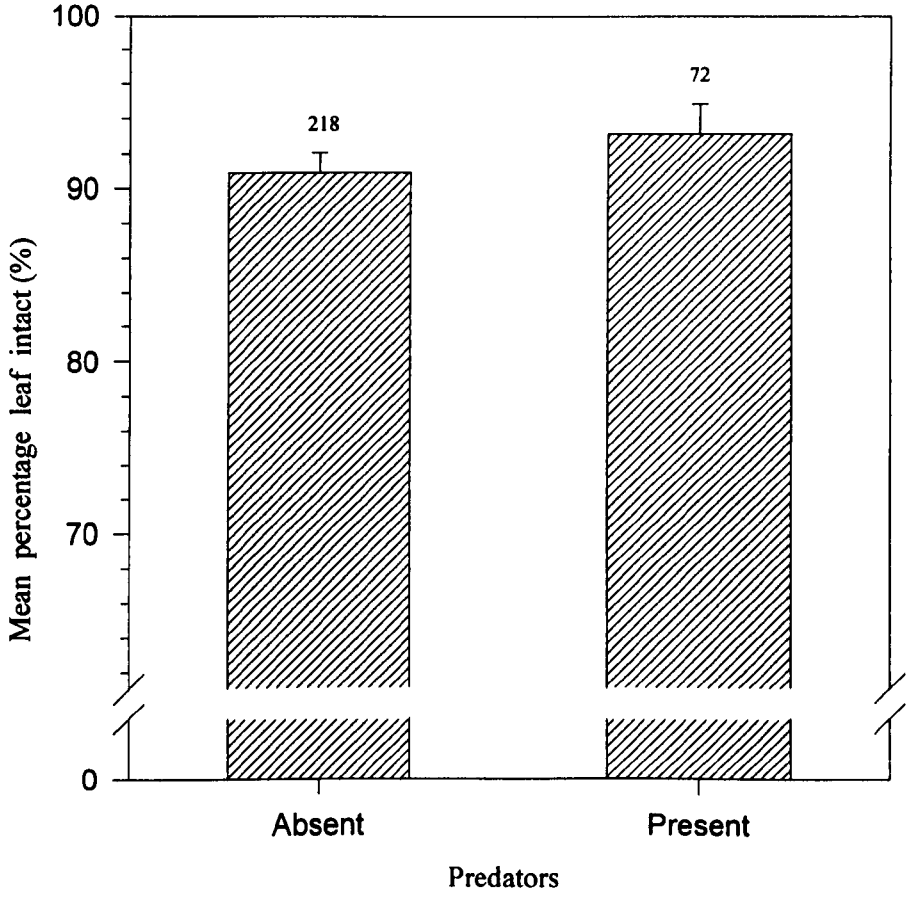
Predators in *Pemphigus* galls on different sized poplar leaf tissue areas



There was no effect of *Populus* x *canadensis* leaf area on predator choice of foraging (ANOVA $F_{1,289} = 0.002$, n.s.).

Figure 7. 3. 6

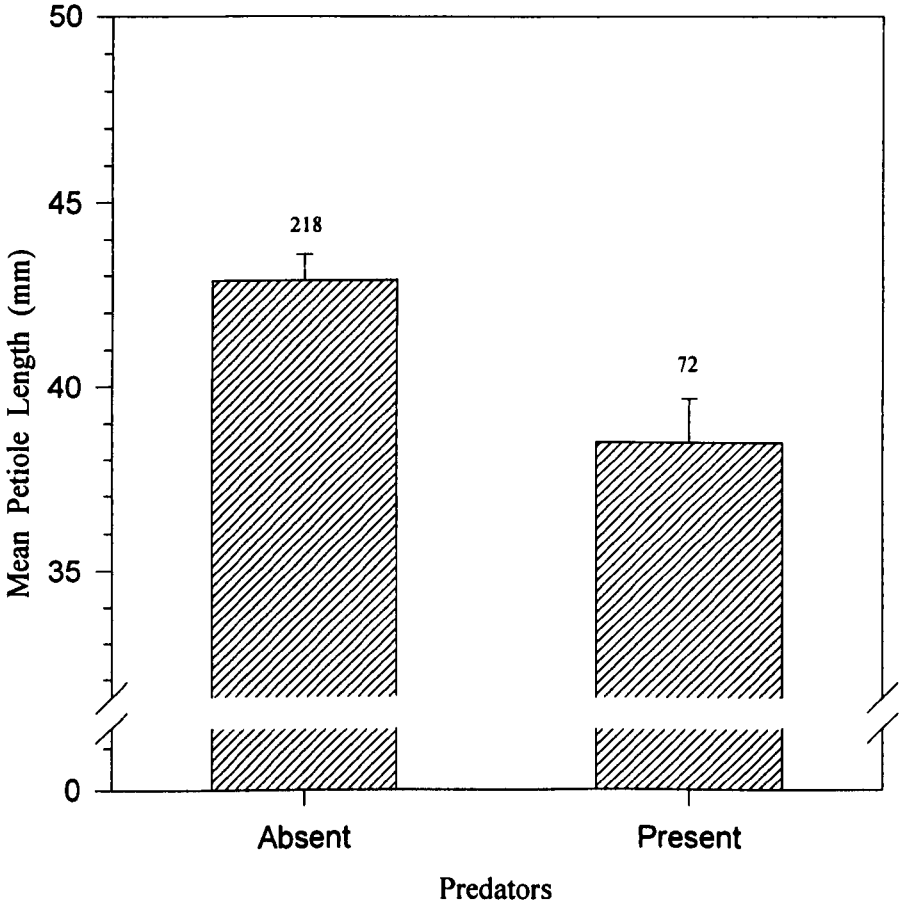
Predators in *Pemphigus* galls on intact poplar leaves



There was no effect of *Populus* x *canadensis* leaf damage on predator choice of foraging (Mann-Whitney U = 7540, n.s., n = 290).

Figure 7. 3. 7

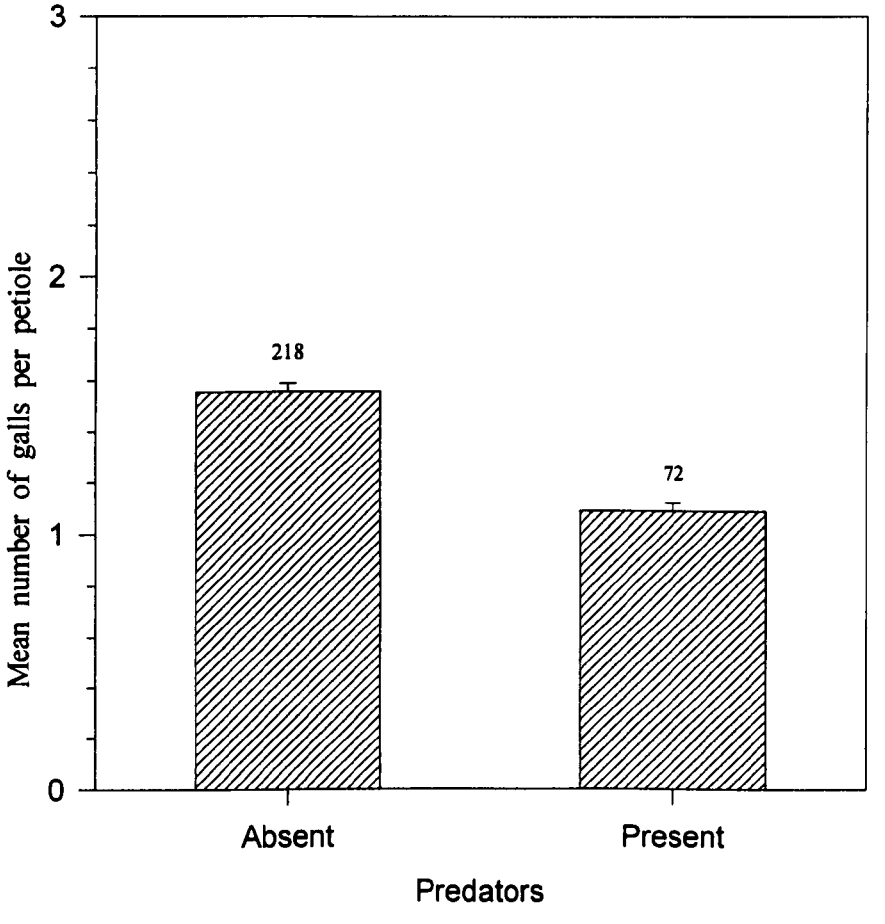
Predators in *Pemphigus* galls on poplar petioles of different lengths



Aphid predators tended to forage in *Pemphigus* galls on smaller *Populus* x *canadensis* petioles (ANOVA $F_{1,289} = 9.387$, $p << 0.01$).

Figure 7. 3. 8

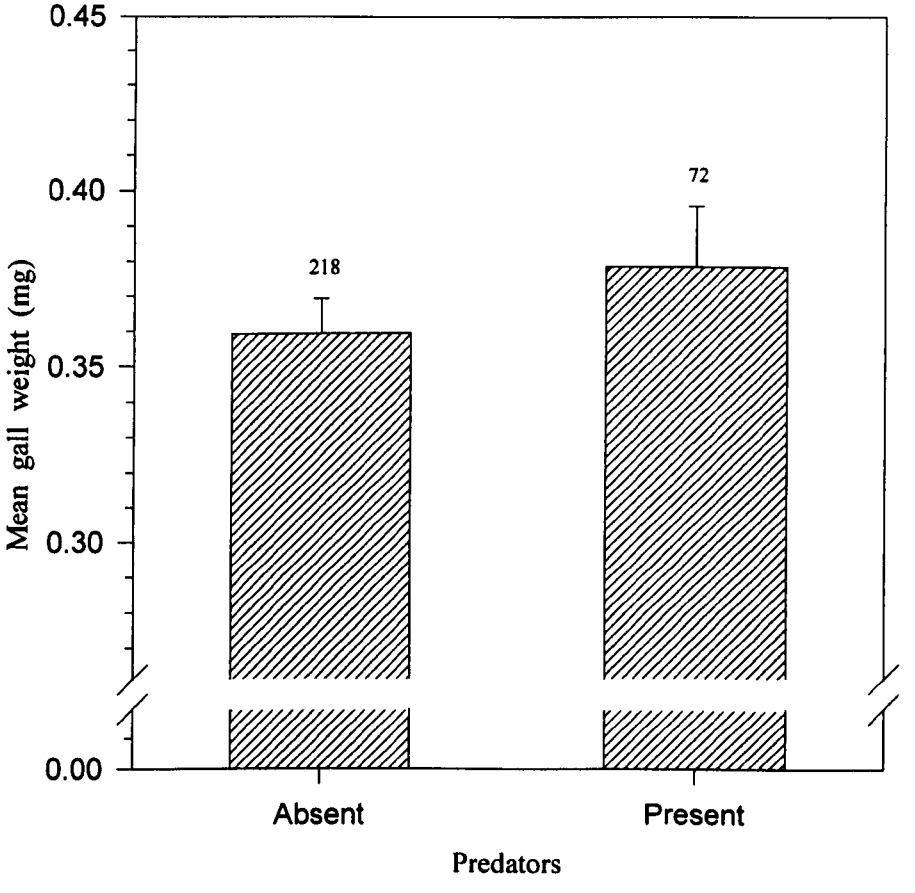
Predators on petioles with one or more *Pemphigus* galls



Aphid predators were more likely to be found on *Populus* x *canadensis* petioles with fewer *P. spyrothecae* galls per petiole (Mann - Whitney U = 4182, $p << 0.001$, n = 290).

Figure 7. 3. 9

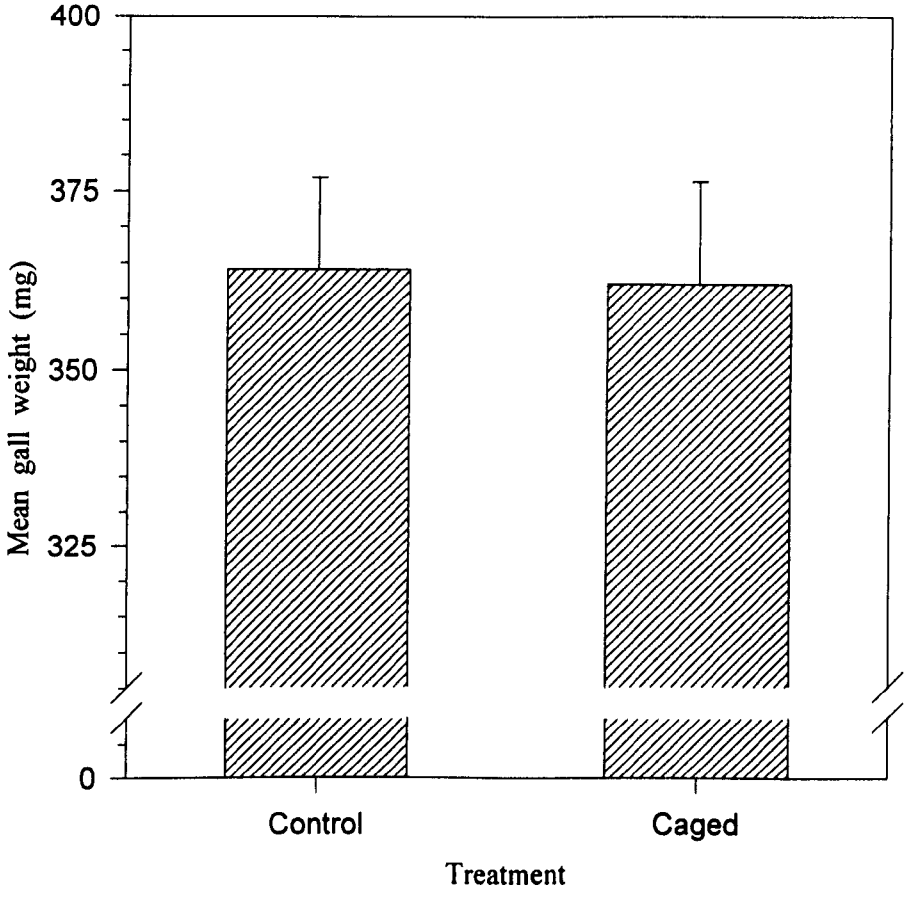
Predation and *Pemphigus* gall weight



Although predators tended to be found at heavier *P. spyrothecae* galls, this was not statistically significant (ANOVA F1,289 = 0.913, n.s.).

Figure 7. 3. 10

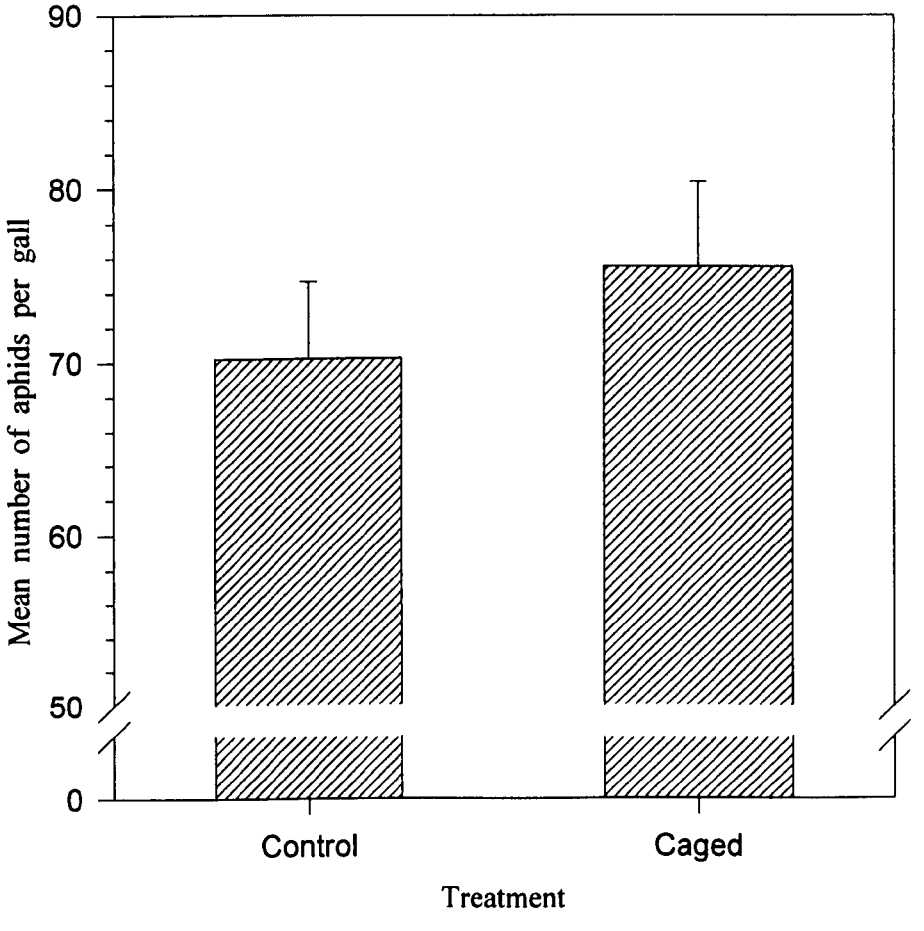
Pemphigus gall weight when gall is enclosed or open



Mean *Pemphigus spyrothecae* gall weights when galls were enclosed in sticky tape or left untouched (control). There was no significant effect of treatment on gall weights (Paired t-test t = 0.15, n.s., n = 132).

Figure 7. 3. 11

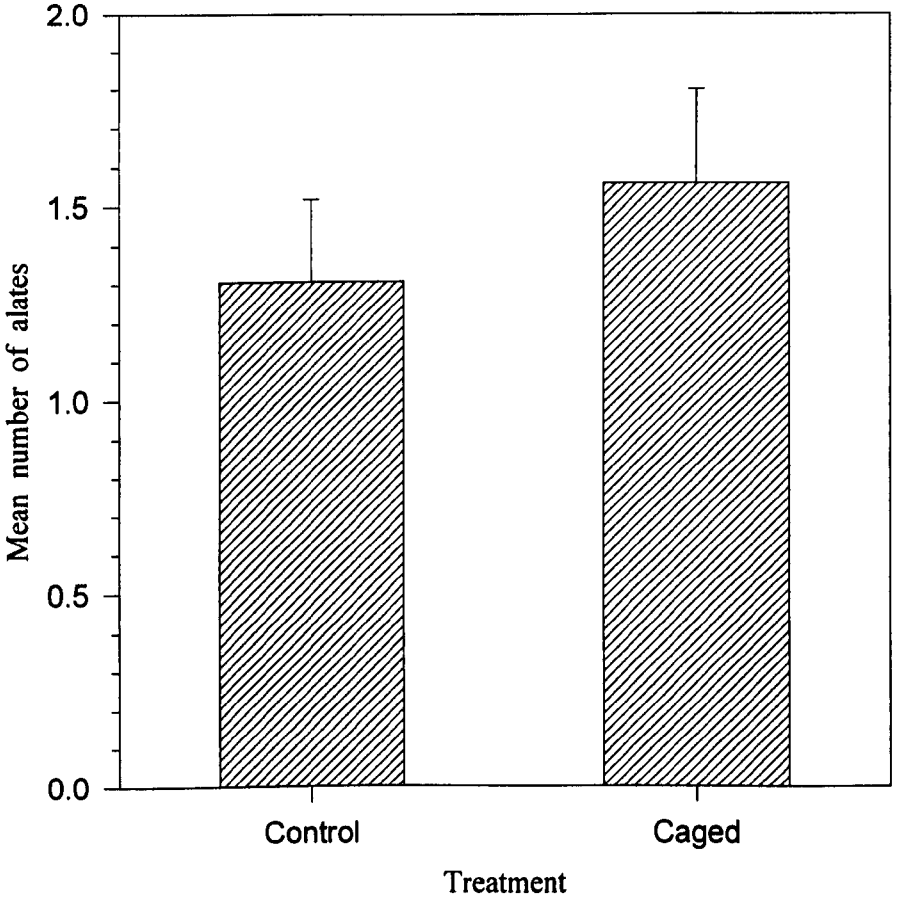
Pemphigus reproductive success when gall is encased or open



Mean number of *P. spyrothecae* aphids per gall when galls were enclosed in sticky tape or left untouched (control). Although the encased galls contained, on average, more offspring, this was not statistically significant (Paired t-test t = 0.904, n.s., n = 132).

Figure 7. 3. 12

Pemphigus alates in encased and open galls



Mean number of alate *P. spyrothecae* when galls were enclosed in sticky tape or left untouched. Although encased galls contained, on average, more alates, this was not statistically significant (Wilcoxon matched pairs test $t_{(\alpha,69)} = -0.783$, n.s.).

Chapter 8

DISCUSSION

When applying natural situations to any of the many habitat selection models based on the theory of ideal free distribution (IFD), the assumptions of equal competitive ability, omniscience, movement without costs and a single type of competition have all been shown to be inappropriate in many situations. Despite these assumptions, in the present study on the population distribution of the galling aphid *Pemphigus spyrothecae*, theories based on unequal competitors are considered the most appropriate models of this system. This study set out to compare between and within patch profitabilitites, and to investigate how competitors and predators affect both patch choice and reproductive fitness of *P. spyrothecae* aphids. My investigations lead me to believe that the most important factor influencing both the choice of galling site and the subsequent success of gall initiation is the availability of closed leaves (leaves not yet unfurled on a shoot) at budburst when the stem mother emerges.

One of the assumptions of the IFD is that habitats should be homogeneous. However, this study adds to the growing evidence of variation in host plant resistance to herbivory. The poplar trees were very variable in their plant structure, e.g. shoot and leaf size (Figs. 2.3.1 and 2.3.3), which resulted in a difference in aphid fitness rates among the trees (Fig. 2.3.9). Even within a single tree, habitat patches (shoots) were heterogeneous, and as predicted, better quality patches conferred a reproductive advantage. Although gall failure was equally likely across all shoot sizes, reproductive success was correlated with shoot size (Fig. 3.2.2). Leaves in the middle part of the shoot were larger than both the apical and basal part of the shoot, and this is where most galls were found (Fig. 3.3.8). Although aphids gained a greater reproductive advantage on larger leaves than on smaller leaves due to leaf area available for photosynthesis (Fig. 3.3.16), the position of the gall on the shoot was not vital (Fig. 3.3.10): aphids are extremely good sinks and efficiently translocate nutrients from adjoining leaves.

Pemphigus spyrothecae stem mothers select their galling sites in spring when they hatch from eggs laid the previous autumn. I predicted therefore that synchrony between host plant budburst and stem mother emergence would be vital in ensuring a successful gall colony. However, whilst there was a significant difference between budburst among the poplar trees, stem mothers emerged within two days on all trees (Fig. 2.3.7). The failure to detect any adaptation to the budburst of individual trees may have been due to the low sample size (n = 13 trees) (Fig.4.3.5).

Poplar leaves only remain supple for a few days, so the time window available for leaf selection is narrow. The IFD assumes that there are no costs involved with movement between patches, but clearly this is unlikely to be the case. Although there are few predators present this early in spring, weather conditions can be extreme. The combination of wind and rain is fatal to many aphid species. Another supposition of models based on the IFD is that foragers have a perfect knowledge of all patches. Again, this is doubtful. Whereas aphids may possibly be able to detect a 'good' patch from a 'bad' patch, there is no evidence to suggest that they can remember patch profitabilities, or are even able to visit all patches to assess their profitability. *Pemphigus spyrothecae* aphids have very little time to explore their environment. Time wasted probing unsuitable sites is costly and could potentially be fatal, if the time window elapses. However, the present study clearly illustrates that *Pemphigus* aphids tend to distribute themselves fairly evenly throughout their environment. I detected no clustering of aphids at any particular distance from the tree trunk (Fig. 4.3.14).

Although I could not detect any micro-adaptation to budburst on host trees, there does appear to be evidence that aphid performance is related to individual trees. Since *P. spyrothecae* is a non-migratory aphid species, this is perhaps not surprising. Although the sexuparae are winged and can no doubt migrate some distance, aphids probably just fly to the nearest trunk, either the one currently on, or a neighbouring trunk. With the exception of aphids from tree D which for some reason did not perform well on clones from any trees, aphids translocated on to plant clones from their own or neighbouring trees attempted to initiate galls more readily than those placed on a clone taken from a tree some distance away (Fig. 4.3.7). They also appeared to settle down

quicker on familiar trees. In this sense the aphids could be regarded as having unequal competitive abilities, since there was a prior experience (whether genetic, biochemical or otherwise) of the resource (as predicted by the model).

The most important factor influencing whether gall initiation was successful appeared to be the availability of petioles with closed leaves (Fig. 4.3.17). When stem mothers emerge in spring the basal and lower middle leaves of many shoots have unfurled, and seem to be unsuitable for galling: in the study year stem mothers either moved away from petioles with unfurled leaves and on to ones with open leaves or died. This may go some way to explaining the distribution pattern of galls on a tree, since the leaves destined to become the largest on the shoot are those most likely to be galled, purely because at colonisation these have yet to be unfurled. The apical leaves on many shoots do not develop until the colonisation period has ended. However, does this mean that stem mothers do not actively exert some choice over their galling site? Many shoots had no galls; ungalled shoots tended to be smaller in size than galled shoots (Fig. 3.3.17). Could this be evidence that stem mothers can assess the potential size of the shoot, perhaps from the turgor pressure of nutrients flowing through the phloem sap? This question could be addressed in a future study.

The presence of other competitors is no doubt one of the most important factors influencing an individuals choice of habitat. The unequal competitors model predicts that an increase in competitor density results in a reduction in fitness: sharing a petiole resulted in smaller galls containing fewer offspring (Fig. 6.3.14). Stem mothers in single galls (accommodating more progeny) were initially, on average, larger than those in galls on shared petioles (Fig.5.3.11), and fundatrix size was also correlated to leaf size (Fig. 5.3.12) (theory predicts payoffs to the various phenotypes will be different). Single galls completed their structural development earlier than either galls on a shared petiole (Fig. 4.3.18), and hence reduced the risk of mortality from adverse weather conditions or predation. Interference may hinder the rate of gall development on doubly-galled petioles, probably through contests for the superior location on the petiole. Although most single galls were located close to the leaf junction (Fig. 3.3.17), galls closer to the point of attachment to the shoot on shared petioles contained, on average, more progeny than their partner galls (Fig. 6.3.12), presumably

by not only intercepting nutrients flowing into the leaf, but also by drawing in resources from neighbouring leaves.

Why aphids reduce their potential fitness by doubling up on a petiole, while other seemingly suitable petioles are ungalled, remains unclear. Stem mother size could, in part, account for this phenomenon. I observed in *Pemphigus spyrothecae* aphids a similar aggressive behaviour to *P. betae*, where larger females usually win the superior galling sites. Another explanation, as mentioned earlier, could be the result of the mechanics of budburst: other leaves on an otherwise appropriate shoot may already have unfurled. However, in many instances, it may be a combination of the above two factors; most doubly-galled petioles occurred on shoots with other galls.

A newcomer on an already occupied petiole may be forced into a trade-off situation with costs involved no matter what the outcome. The aphid can either stay on the petiole thereby reducing its reproductive success and perhaps in the process be compelled to engage in a competitive interaction with the resident aphid, or face the uncertain move to another site and possibly die in the attempt. It is interesting to note that three quarters of all gall failures were alone on the petiole: staying put on a shared petiole may not be such a bad option (theory predicts that no individual will be able to increase its payoff by shifting to another patch). If the petiole is already occupied by other stem mothers, this may, perhaps in some biochemical way, enhance the petiole as a galling habitat. The combined sink effect of two galls may compensate to some extent the shared use of resources.

Predators are known to regulate the population density of many herbivores, but the exact clues to guide predators to insects such as galling aphids, especially cryptically coloured galls, remain unclear. There have been reported instances where natural enemies were attracted to particular sized galls. Here, on average, larger galls appeared to be slightly more vulnerable to attack (Fig. 7.3.9), and predators appeared to forage in an inverse density-dependent manner (Fig. 7.3.8). Apart from being encased within a gall, the aphids have no means of evading predators, therefore the evolution of a soldier caste is perhaps not surprising. The production of soldiers occurs within the gall, and the soldiers not only defend the colony against predators,

but also maintain the health of the colony through house cleaning duties. My results suggest that soldiers enhance the survival of the gall colony.

The current theories can be useful tools to predict population distributions. However, it is important to bear in mind that contradictions to the assumptions of these models such as lack of omniscience and costs to movement also affect predicted distributions, tending to lead to greater use of poorer patches. Because there is no predetermined relationship between competitor density and the strength of interference, different models assume different relationships between these factors and produce correspondingly different results. Further experimental work is required to demonstrate the relationships between factors such as competitor density, resource distribution and intake rates, since current models are based largely on theory rather than observation. Although different forms of competition often occur together in nature, the present theory tends to consider one or other in isolation, so further theoretical and practical work would be of use too. It is difficult to predict whether a 'grand unified theory' of animal distribution will ever be feasible since it is not easy to assess the relative importance of the numerous factors involved. However, the development of individual level models is a step in the right direction, and has provided scientists with an awareness of short term population dynamics.

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